IN VITRO EVALUATION OF TEBUCONAZOLE ON *Magnaporthe oryzae triticum* TO CONTROL BLAST OF WHEAT: IN PROCESSES OF REACTIVE OXYGEN SPECIES GENERATION AND ANTIOXIDANT DEFENSE RESPONSES

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This is to certify that the thesis entitled, ***In Vitro* Evaluation of Teboconazole on *Magnaporthe oryzae triticum* to Control Blast of Wheat: In Processes of Reactive Oxygen Species Generation and Antioxidant Defense Responses** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN PLANT PATHOLOGY* embodies the results of a piece of bona fide research work carried out by **JAFRIN JAHAN JERIN** bearing Registration No**. 21-010088** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged.

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TO

MY BELOVED PARENTS AND

TEACHERS

ABSTRACT

Blast is an important disease of wheat that significantly reduces the plant growth and productivity. Proper management can ameliorate dysfunction in and improve the plant growth and productivity. Therefore, the present study was conducted to explore the protective role of the tebuconazole fungicide in helping wheat seedlings to tolerate blast disease. Ten-day-old hydroponically grown seedlings were allowed to inoculation of *Magnaporthe oryzae triticum* (MoT) spore suspension and with the tebuconazole fungicides (0.3 g L^{-1}) for the next six days. The inoculation of MoT spore suspension deteriorated the growth of the wheat seedlings by increasing the number of infected plant and leaf lesions. Among the varieties, lower blast symptom was observed in BARI Gom 33 (V2P) compared to BARI Gom 26 (V1P). The malondialdehyde (MDA) content increased by 102.80 and 163.96% in BARI Gom 26 and BARI Gom 33, respectively compared to control. Shoot and root electrolyte leakage also increased by MoT inoculation. Therefore, the level of $H_2O_2$and $O_2^{\bullet-}$ also increased that observed in histochemical detection. The infection of blast disease, relative water content decreased by 50.18 and 51.26% in BARI Gom 26 and BARI Gom 33, respectively compared to control plant. Photosynthetic pigment such as Chl *a*, Chl *b*, Chl *(a+b*), and carotenoid contents also reduced by blast infection. Thus the growth and biomass of wheat seedlings were decreased. However, tebuconazole fungicide reduced the oxidative damage by reducing MDA, electrolyte leakage, $H_2O_2$and $O_2^{\bullet-}$ production. Therefore, improved relative water content and photosynthetic pigments as well as improved the growth and biomass of wheat seedlings. These results indicate the exogenous application of tebuconazole fungicide is a promising approach to improve blast tolerance in wheat plants. Further investigation is needed under field conditions to determine the blast-tolerance induced by tebuconazole fungicide application.

Keywords: Blast, *Magnaporthe oryzae triticum*, Wheat, Oxidative stress, Tebuconazole

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The Author

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$^{\bullet}OH$	:	Hydroxyl radical
$^1O_2$	:	Singlet oxygen
ABA	:	Abscisic acid
ANOVA	:	Analysis of variance
AO	:	Ascorbate oxidase
APX	:	Ascorbate peroxidase
AsA	:	Ascorbic acid/ascorbate
AUMGC	:	Area under mycelia growth curve
BAC	:	Biological accumulation coefficient
BCF	:	Biological concentration factor
BSA	:	Bovine serum albumin
Car	:	Carotenoid
CAT	:	Catalase
Cd	:	Cadmium
CDNB	:	1, Chloro-2,4-dinitrobenzene
Chl	:	Chlorophyll
CRD	:	Completely randomized design
DAB	:	3,3′-diaminobenzidine
dH_2O	:	Distilled water
DHA	:	Dehydroascorbate
DHAR	:	Dehydroascorbate reductase
DMI	:	Demethylation inhibitors
DTNB	:	5,5'-Dithio-bis (2-nitrobenzoic acid)

DTT : Dithiothretitol

DW : Dry weight

EC : Electrical conductivity

EDTA : Ethylenediaminetetraacetic acid

EL : Electrolyte leakage

EtAc : Ethyl acetate

FAO : Food and Agriculture Organization

FAOSTAT : Food and Agriculture Organization Corporate Statistical Database

FW : Fresh weight

Gly–I : Glyoxalse–I

Gly–II : Glyoxalse–II

GPX : Glutathione peroxidase

GR : Glutathione reductase

GSH : Reduced glutathione

GSSG : Oxidized glutathione

GST : Glutathione *S*-transferase

H_2O_2 : Hydrogen peroxide

H_2SO_4 : Sulfuric acid

HO_2^- : Peroxide ion

$HO_2^{\bullet}$: Peroxide radical

IAA : Indoleacetic acid

Ip : Ipomeamarone

IpOH : Ipomeamaronol

LOX : Lipoxygenase

LSD : Least Significant Difference

MBC : Methyl benzimidazole carbamate

MDA : Malondialdehyde

MDHA : Monodehydroascorbate

MDHAR : Monodehydroascorbate reductase

MG : Methylglyoxal

MoT : *Magnaportheoryzaetriticum*

NADPH phosphate : Nicotinamide adenine dinucleotide

NBT : Nitroblue tetrazolium chloride

NTB : 2-Nitro-5-thiobenzoic acid

$O_2^{\bullet-}$: Superoxide anion

PAs : Polyamines

PBZ : Paclobutrazol

PDA : Potato dextrose agar

POD : Peroxidase

POX : Peroxidases

PPO : Polyphenol oxidase

PRO : Propiconazole

PRX : Peroxiredoxins

PSA : Potato sucrose agar

QoIs : Quinone outside inhibitors

ROS : Reactive oxygen species

RWC	:	Reactive water content
SDHI	:	Succinate dehydrogenase inhibitor
SLG	:	*S*-D-Lactoylglutathione
SOD	:	Superoxide dismutase
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
TEB	:	Tebuconazole
TF	:	Translocation factor
$TiCl_4$	:	Titanium tetrachloride
TRI	:	Trifloxystrobin
TRX	:	Thioredoxin
TW	:	Turgid weight
UV	:	Ultra violet
WB	:	Wheat Blast

CHAPTER I

INTRODUCTION

The population of the globe has increased dramatically during the past century; it was predicted to reach 9.8 billion people in 2050 after crossing 7.3 billion people by the middle of 2015. Due to the world's vast population, there will be an increase in food demand, which is predicted to reach 14.88 billion tons in 2050 (Islam and Karim, 2019). As a result, food security will be a major concern for many nations, especially developing nations. Plants must deal with a wide range of intricate interactions involving many different environmental elements. Plants exposed to biotic and abiotic stress experience a change in metabolism, which eventually reduces fitness and productivity (Shao *et al*., 2008). Abiotic stresses such as drought, temperature, radiation, salinity, heavy metals/metalloid and nutrient deprivation cause physiological and metabolic disorders and adversely affect plant phenological and developmental processes. Biotic stress, one of the environmental stresses, is a serious problem that drastically reduces crop yields. The ability of the plants to develop their full genetic potential for vegetative and reproductive growth is hampered by biotic factors such as microbial diseases, weeds, and herbivores (Qaim, 2011; Ashraf *et al*., 2012). Plants, which are sessile, have developed an abundance of defense mechanisms that allow them to recognize certain challenges and respond by activating complex signaling networks that bring about the necessary biochemical and physiological changes to overcome the stress (Atkinson and Urwin, 2012; Lamers *et al*., 2020).

The most significant crop for the human population is wheat (*Triticum aestivum* L.) which is consumed worldwide.Wheat comes in third place behind maize and rice in terms of total cereal production (Igrejas and Branlard, 2020). The top three producers were China, India, and Russia, with production of 134, 98.5, and 85.9 MT, respectively, in 2017, according to the Food and Agriculture Organization (FAO). Additionally, there were over 220 M hectares of cultivated land in 2017 (FAO 2018). The Department of Agricultural Extension (DAE) of Bangladesh reports that the total area of wheat grown in blast-affected areas was 99,259 hectares in 2015–16 and 47,278 hectares in 2016–17 (Islam *et al.* 2019).

One of the most pervasive and severely debilitating crop diseases is rice blast, and the pathogen that causes it, *Magnaporthe oryzae* pathotype *Oryza* (MoO), is placed first among the ten most dangerous fungal plant pathogens (Dean *et al.*, 2012). In contrast, wheat blast (WB) is far less well-known, having only ever been found in South America up until that point.Wheat blast (WB), in contrast, is far less well-known and was only found in South America for thirty years prior to its current outbreak in Bangladesh (Ceresini *et al.*, 2018). *M. oryzae* is the pathogen that causes both the rice and wheat blasts, despite initial assumptions to the contrary. Although the two pathotypes of *M. oryzae* have identical physical features, MoT is genetically distinct from MoO and is the cause of wheat blast (WB) disease (Cruz and Valent, 2017). In comparison to rice blast, WB has received far less scientific attention due to its small geographic pandemic areas. After scientists had issued a warning that the disease might spread to other continents (Duveiller *et al.*, 2011), it was subsequently reported in Bangladesh, in Asia and Zambia in Africa (Malaker *et al.*, 2016; Tembo *et al.*, 2020). Since then, WB has attracted more and more attention due to the possibility of it spreading to nearby nations like India, Pakistan, and China, who are all important producers of wheat and where wheat is one of the main staple foods for billions of people. The strong genetic similarity between Bangladeshi MoT isolates and those from South America was confirmed by molecular analyses using MoT-specific markers and comparative genome sequencing (Islam *et al.*, 2016; Malaker *et al.*, 2016). As much as 100% of yield losses have been known to result from WB (Duveiller*et al.*, 2016a; Cruz and Valent, 2017). Therefore, action is required to prevent MoT from spreading to other regions of the globe because failure to do so could result in a disaster. In the recent years, WB has been the subject of active research and breeding study. Countless research articles have been written on every element of WB research, and numerous WB-resistant cultivars have been released in WB-affected or WB-threatened nations. Wheat blast disease, which is brought on by the *Magnaporthe oryzae Triticum* (MoT) pathotype, has become a serious danger to wheat productivity in Bangladesh. As was previously noted, there have been more blast disease outbreaks recorded in Bangladesh in 2016 (Islam *et al*. 2016).

A major symptom of the disease is spikes that have been bleached (dead) entirely or in part. The spike had either no grain or shriveled grain as a result of full or partial bleaching. No grains were produced as a result of head infections during the flowering stage, but pale, tiny, shriveled grains were produced during the grain filling stage. With up to 100% production losses, the pandemic spread to an estimated 15,000 hectares, or 16% of Bangladesh's wheat-growing area. At the reproductive stage of the crop, the disease's first observable sign is seen in a smattering of wheat fields. Over time, the patches combine, gravely damaging the entire field. While the foliage may still be green, the spikes in the infected area take on a silvery hue; however, the most recognizable symptom is seen on the spikes (Singh, 2017; Cruz *et al*., 2015; Malaker *et al*., 2016; Saharan *et al*., 2016; Cruz and Valent, 2017). The fungus MoT can infect all above-ground parts of wheat, including spike, leaf, peduncle, glume, awn, and seed. The most noticeable signs of wheat blast are partially or completely bleached spikes, which begin at an apparent blackish-gray infection point at the rachis or base of infected spikes. Partial or complete drying occurs depending on where the infection is located on the spike. Under conditions of high inoculum pressure, it is occasionally possible to see many foci of infection in a single rachis in susceptible cultivars. The plant's mechanism for transporting nutrients can be disrupted by an infection in the rachis or peduncle, which will eventually cause harm to all of the upper spikelets above the infection spots (Cruz and Valent, 2017). Highly susceptible cultivars show gray, dark-gray, or black sporulation of the fungus at the site of rachis infection (Igarashi, 1990; Islam *et al*., 2016). While infected glumes have elongated lesions with reddish brown to dark gray edges and a white to light brown center, infected awns exhibit a brown to white stain (Saharan *et al*., 2016; Cruz and Valent, 2017). Lesions contain gray centers during sporulation, which become white to tan when spores are released (Igarashi *et al*., 1986; Igarashi, 1990). Wheat blast damage to grains varies in severity and timing depending on the infection. The absolute sterility of spikes caused by an infection that occurs before anthesis or at the beginning of blooming leads to seed abortion (Goulart *et al*., 1990; Goulart and Paiva, 1992; Urashima *et al*., 2009). Small, wrinkled, malformed, and low test weight kernels which are unfit for human consumption are the outcomes of infection at the grain filling stage (Goulart *et al*., 2007; Malaker *et al*., 2016).

A fungal infection results in oxidative stress in plants because it produces more ROS, while different chemical and physical methods can induce oxidative damage in fungi by producing more ROS. As a result, ROS-induced oxidative damage results in increased cell membrane permeability, the disruption of cellular components, and the inhibition of protein synthesis and enzyme activity. Antioxidants, on the other hand, prevent auto-oxidation by limiting the synthesis or mobility of ROS, and living organisms have the capacity to build antioxidant defense mechanisms to detoxify ROS. Self-defense mechanisms are insufficient for ROS detoxification under extreme stress circumstances, necessitating exogenous measures to strengthen antioxidant defense mechanisms. Numerous biotic stressors, including viruses and herbivores, substantially reduce crop output during growth by changing a variety of biochemical, physiological, and metabolic processes.Among them, a very important signaling reaction against pest and disease attack is the generation of ROS.The plant's metabolic pathways are redirected to safeguard plant equipment and minimize damage as a result of the ROS, which serves as an alert. In chloroplasts, mitochondria, and peroxisomes, ROS is largely produced. But under stress, it comes from secondary locations such the cell wall, endoplasmic reticulum, and apoplast. The increased ROS production brought on by the signaling during the stress damages plant cells by generating a redox imbalance, lipid peroxidation, and the oxidation of proteins, nucleic acids, and chlorophyll. Plant scavenging processes limit the damaging effects of high ROS.

On the other hand, pathogen infections result in the activation of certain ROS-producing enzymes, such as NADPH oxidases or cell wall PODs (Mittler, 2002). The oxidative burst, which is caused by these enzymes' excessive production of ROS, is well-known (Heller and Tudzynski, 2011). According to Zurbriggen *et al*. (2010), the HR, which inhibits the pathogen from spreading to neighboring tissue, may be brought on by an oxidative burst in plants. Inhibiting further pathogen growth and disease progression, the HR can also function as a critical signal to activate many pathways controlling plant defensive responses and phytohormone synthesis (Beers and McDowell, 2001). Sequential activation of these pathways alters the expression of genes involved in the plant defense system, triggers the production of phytoalexins, and results in the deposition of callose. As a result of all of this, a pathogen develops a resistance response (Forman *et al*., 2010).

For the management of plant growth, stress, and disease, triazole-based plant growth regulators are widely employed. They alter a variety of biotic and abiotic stresses-related molecular, physiological, and biochemical processes in plants to promote growth and induce tolerance. For their aforementioned function in plants, they often regulate the endogenous levels of phytohormones and other substances including gibberellins, cytokinins, auxins, abscisic acid, and ethylene. Triazolic chemicals have attracted a lot of attention recently due to efforts to minimize their ecotoxicological effects and increase their efficiency.The theoretical foundation of sustainable agriculture is strengthened by the controlled release triazole formulations based on nanocarriers. The discovery of novel triazoles that target strigolactones to regulate growth and induce stress tolerance in plants has recently undergone a target-oriented shift. Here, we highlight several significant recent advancements in the use of triazoles for stress management and plant growth regulation.

Higher temperatures (18–30°C) and high humidity (plants moist for at least 10 hours owing to rain or dew fall) are conducive to the development of wheat blast disease (Cardoso *et al*. 2008). Chemical fungicides can effectively and conveniently stop microbial growth when applied exogenously. In order to prevent the disease, a foliar spray of triazoles and strobilurin fungicides has been recommended (Kohli *et al*. 2011). However, persistent use of related fungicides may cause pathogens to acquire resistance to a specific fungicide. For instance, from 2005 to 2012, MoT's resistance to strobilurin fungicides grew by 36% to 90% (Castroagudn *et al*. 2015). At the right concentration, many triazole drugs such paclobutrazol, tebuconazole and hexaconazole may be beneficial in controlling wheat blast disease. So, it isrequied to identify the mechanisms by which tebuconazole in wheat plants suppress blast disease. The food and nutritional security of the world's growing population is seriously threatened by the rising rate of new blast diseases in wheat plants. If we can develop an effective management approach for not only suppressing the pathogen but also promoting the plant growth , it could be a great initiative for controlling the wheat blast disease.Therefore, the primary goal of the suggested study is to examine the antioxidant defense mechanisms in the wheat plant and MoT following the application of tebuconazole fungicide.

Keeping these facts in mind the study is undertaken to achieve the following objectives:

- To measure the growth morphology and oxidative damage in *Magnaporthe oryzae triticum* inoculated wheat plants
- To investigate the roles of tebuconazole fungicide on physiology and antioxidant defense systems for improving blast disease tolerance in wheat plants

CHAPTER II

REVIEW OF LITERATURE

One of the most significant crops for human nutrition is wheat, which accounts for 17% (or one sixth) of all cropland, feeds over half of the world's population and 20% (or one fifth) of all food calories and protein. Although wheat production has increased significantly over the past four decades, there has been a slowdown in recent years, which has resulted in the lowest current levels of global wheat reserves since 1948/49. Contrarily, it is predicted that, without any new land becoming accessible for this crop, wheat grain output must expand at a pace of 2% year in order to fulfill growing human requirements. A new level of comprehension of the structure and functionality of the wheat genome is needed to meet this challenge. Wheat blast, often known as "brusone," is a recent fungal disease brought on by the *Magnaporthe oryzae triticum* (MoT) pathotype, also known as *Pyricularia oryzae*. The disease was initially identified in the Brazilian state of Paraná in 1985 (Igarashi *et al*., 1986), and it most likely first appeared as a "host jump" from a nearby grass (Castroagudin *et al*., 2017; Inoue *et al*., 2017). The wheat blast pathogen quickly spread from Paraná to the nearby states of Sao Paulo, Mato Grosso do Sul, the Rio Grande do Sul, Minas Gerais, Goias, and Brasilia in 1993 (Igarashi, 1991; Prabhu *et al*., 1992; Anjos *et al*., 1996). In South America's wheat-growing regions, the disease then progressively spread until it reached eastern Bolivia in 1996, eastern Paraguay in 2002, and northern Argentina in 2007. For many years, the illness has been a major impediment to wheat yield, especially in Brazil's middle Cerrado region, where the disease is more likely to grow due to the region's humid, subtropical climate. Wheat blast was first discovered outside of South America in the United States in 2011 on a single plant in Princeton, KY, and it was believed that this outbreak was caused by an endemic pathogen that infected Lolium rather than an exotic introduction from South America (Farman *et al*.,2017). Later, Bangladesh reported the first 2016 occurrence of wheat blast outside of South America. The illness is thought to have arrived in Bangladesh via Brazilian imports of wheat grain. The wheat blast was discovered in Africa in 2018 during the rainy season in the Zambian rainfed wheat farming system. The experimental plots and farmer-grown wheat fields at Malashi in the Mpika region of Muchinga Province were significantly affected by the wheat blast (Tembo *et al*., 2020). Although the cause of the disease in Zambia is unknown, seeds tainted with pathogens may have aided in its spread to these areas. The Bangladesh outbreak is the biggest since the initial epidemic in 1985. Approximately 3.5% of Bangladesh's total wheat acreage was affected by the pandemic, which was widespread in the southern districts such as Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, and Bhola (Islam *et al*., 2016; Malaker *et al*., 2016). Due to its ability to spread to other wheat-growing regions, the wheat blast is now considered a severe concern in some of the newly affected areas where it has become a permanent issue. This terrible disease, known as the wheat blast, can damage up to 100% crop loss under favourable conditions. Due to a major wheat blast epidemic, Bolivia experienced a 69% crop loss in 1997 (Mottaleb *et al*., 2018). According to Duveiller *et al.* (2016), the wheat blast has recently caused crop losses of 10–100% in South America's Southern Cone. According to Islam *et al*. (2016), the Bangladeshi wheat blast outbreak in 2016 reduced wheat yield by 5–51% in the afflicted districts. With such lethal potential, a widespread outbreak of the illness might jeopardize global food security.

2.1 Causal organism of the deadly blast

Magnaporthe oryzae, formerly known as *M. grisea* is the causal agent of the blast. The fungus is a species complex that infects over 50 grass species, including rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), perennial and annual ryegrass (*Lolium* sp.), finger millet (*Eleusine coracana*), Italian (foxtail) millet (*Setaria italica*), and crabgrass (*Digitarias anguinalis* L.). The fungus was named *Pyricularia* after the pyriform shape of the asexual conidia of *P. grisea* on crabgrass (Saccardo,1880). The rice isolates were later classified as *P. oryzae*

(Cavara, 1892). The hosts of *Pyricularia* species assigned *P. oryzae* to isolates of rice and *P. grisea* to isolates of all other cereals and grasses (Sprague, 1950). Based on the shape of the ascospores, the sexual form of *P. grisea* from *Digitaria* was discovered in the laboratory and given the name *Magnaporthe grisea* (Barr, 1977; Couch and Kohn, 2002). The isolates from *Oryza, Setaria, Panicum, Eleusine, Triticum, and Lolium* form a genetically close, interfertile group (the CC crop isolate group), different from the crabgrass isolates originally designated *P. grisea,* according to extensive analysis of the pathogenicity, mating compatibility, and RFLPs of *Pyricularia* isolates from a variety of hosts (Kato *et al*., 2000; Tosa et al. They suggested changing the name of the CC group to *P. oryzae*. The tight connection of agriculturally relevant CC isolates was confirmed by Couch and Kohn (2002) using a multilocus phylogenetic analysis. These pathogens were then classified into the separate species *M. oryzae,* while *M. grisea* was maintained for isolates pathogenic to *Digitaria* species. The frequent use of both *Pyricularia* and *Magnaporthe* led to a problem for blast researchers when it was decided in 2011 that each fungus should have a distinct name. Therefore, both *Magnaporthe* and *Pyricularia* will continue to be used going forward, as has been decided by the community (Zhang *et al*., 2016). *Pyricularia graminis tritici,* a new species created lately by combining a subset of the wheat pathogen population with pathogens from finger millet and other grasses, divides the wheat blast population into two species (Castroagudin *et al*., 2016). The fungus's asexual conidia have a pyriform shape and can be hyaline or light gray in color (Cruz and Valent, 2017). The three cells in each of the conidia have the same nucleus. According to Cruz and Valent (2017), the fungus' sexual form is a Pyrenomycete that typically produces four-celled ascospores in asci that are randomly placed within long-necked perithecia. The mating compatibility of fully fertile strains, which are hermaphrodites and self-sterile, is regulated by different alleles of the mating-type locus MAT1. Highly fertile hermaphrodite strains mate as females and males in crosses with hermaphrodite strains of the opposite mating type at a temperature of 20°C and in the presence of light. A Phialophora-like anamorph is also produced by sexually active *M. orzyae* strains, in which phialides are transformed into tiny, crescent-shaped microconidia (Chuma *et al*., 2009). The purpose of these microconidia in nature is unknown, despite the fact that they germinate at low levels and infect plants through wounds (Zhang *et al*., 2014). Conidia and ascospores both germinate and produce appressoria on hydrophobic surfaces. For purposes of penetrating and colonizing the host leaf surface, appressoria that develop in water droplets, such as dew, produce exceptionally high turgor pressure. According to Araujo *et al*. (2016), fungal strains usually failed to penetrate non-adapted hosts and, when they did, they caused cytoplasmic granulation or hypersensitive-like reactions that could have resulted from gene-for-gene exchanges.

2.2 Production losses

In warm, humid wheat growing zones, wheat blast is one of the most damaging and yield-limiting diseases. Due to the disease's significant reductions in grain output and quality, it has economic significance (Goulart *et al*., 2007). The spike infection that causes the most yield loss is during anthesis or the early stages of grain filling (Goulart *et al*., 2007) and/or when the fungus attacks at the base of the spike, preventing the growth of the grains and entirely killing the head (Kohli *et al*., 2011). When a susceptible cultivar is produced in Bangladesh under late-sown conditions and in South America under early-sown conditions, yield losses can reach up to 100% (Roy et al., unpublished; He *et al*., 2020). The severity of the illness, genotype, crop growth stage, planting date, weather conditions (temperature, humidity, rainfall, etc.), and losses resulting from the disease all affect these factors (CIMMYT, 2016; Cruz and Valent, 2017). According to Duveiller *et al*. (2016), the losses in grain output in South America were estimated to be between 10 to 100%. According to Goulart and Paiva (1992), yield losses in 1987 ranged between 10.5 and 53% in three Brazilian states (Parana, Matto Grosso do Sul, and Sao Paulo), which led some farmers to forego growing wheat (Callaway, 2016).

The disease again decimated the early-planted crops the following year (1997), resulting in a 100% yield loss, which caused a dramatic fall in Bolivia's production of wheat in the following years. Production losses of more than 70% were noted in the early publicized fields in Paraguay, when the first outbreak occurred in 2002 (Viedma and Morel, 2002). The majority of the harvested grain had to be used as animal feed since it did not fulfill marketable test weight requirements. According to estimations by the Department of Agricultural Extension, the total yield loss in Bangladesh in 2016 was close to 50% in the 15,000 hectares affected (Islam *et al.*, 2016). This constituted a serious danger to the nation's total wheat production.The disease reappeared in the subsequent years (2017–2020) with comparatively lower disease severity, and an insignificant yield loss (1–5%) was incurred because of unfavorable weather conditions and the adoption of different management packages.

2.3 Pathogen biology

A haploid, filamentous, ascomycetous fungus known as *Magnaporthe oryzae* B.C. Couch and L.M. Kohn (anamorph *Pyricularia oryzae Cavara*) is the cause of wheat blast (Couch and Kohn, 2002). The fungus can only reproduce sexually when two sexually compatible and fertile individuals cross, due to its self-incompatibility (Maciel *et al.*, 2014; Maciel, 2019). When the male benefactor's compatible nucleus or nuclei are able to enter the female receptive structure known as the ascogonium via conidia or receptor hyphae, this occurs (Kang *et al.*, 1994; Moreira *et al.*, 2015). The fungus is extremely host-specific and is unable to infect hosts that are not compatible. *M. oryzae* isolates are categorized into various pathotypes based on host specialization, mating style, and genetic similarity (Urashima *et al.*, 1993; Kato *et al.*, 2000; Tosa *et al.*, 2004; Tosa and Chuma, 2014). As for the pathotypes, *Oryza* causes infection in rice, *Setaria* in foxtail millet, *Eleusine* in finger millet, *Panicum* in proso millet, *Triticum* in wheat, *Avena* in oat, *Lolium* in perennial and annual ryegrass, and many others in grasses (Kato *et al.*, 2000; Farman, 2002; Tosa *et al.*, 2004; Maciel, 2019). Based on host range (Prabhu *et al.*, 1992; Urashima *et al.*, 1993), sexual fertility (Urashima *et al.*, 1993), and DNA fingerprinting (Urashima *et al.*, 1999; Urashima *et al.*, 2005), it has been demonstrated that MoT is unique from other host-specific pathotypes. According to Tosa *et al.* (2006), isolates from each host are completely pathogenic to that host's genus. The pathotypes mentioned above are interfertile and genetically close to the *Digitaria* isolates that were initially given the *P. grisea* designation (Urashima *et al.*, 1993; Kato *et al.*, 2000; Murakami *et al.*, 2000; Tosa *et al.*, 2004, 2006), which was later confirmed with a multilocus phylogenetic analysis (Kato *et al.*, 2000; Couch and Kohn, 2002). Notably, MoT targets not only wheat but also triticale, barley, and durum, which are relatives of wheat (Roy *et al.*, 2020, 2021). On either of the alternate hosts, there was no cross-infection between rice and wheat blast isolates (Prabhu *et al.*, 1992; Tosa *et al.*, 2004). The genetic diversity of the Triticum pathotype population is larger than that of other pathotypes due to its rapid evolution (Urashima *et al.*, 2005; Tosa *et al.*, 2006; Maciel *et al.*, 2014; CIMMYT, 2016).

2.4 Symptomatology of wheat blast

In a sporadic patch of a wheat field, the disease's first observable symptom is seen during the crop's reproductive stage. Over time, the patches combine, gravely damaging the entire field. While the foliage may still be green, the spikes in the infected area take on a silvery hue (Singh, 2017, Cruz *et al.*, 2015; Cruz and Valent, 2017); however, the most recognizable symptom is seen on the spikes (Malaker *et al.*, 2016; Saharan *et al.*, 2016; Cruz and Valent, 2017). The fungus MoT can infect all above-ground parts of wheat, including spike, leaf, peduncle, glume, awn, and seed. The most noticeable signs of wheat blast are partially or completely bleached spikes, which begin at an apparent blackish-gray infection point at the rachis or base of infected spikes. Partial or complete drying occurs depending on where the infection is located on the spike. Under conditions of high inoculum pressure, it is occasionally possible to see many foci of infection in a single rachis in susceptible cultivars. The plant's mechanism for transporting nutrients can be disrupted by an infection in the rachis or peduncle, which will eventually cause harm to all of the upper spikelets above the infection spots (Cruz and Valent, 2017). Highly susceptible cultivars show gray, dark-gray, or black sporulation of the fungus at the site of the rachis infection (Igarashi, 1990; Islam *et al*., 2016). While infected glumes have elongated lesions with reddish brown to dark gray edges and a white to light brown center, infected awns exhibit a brown to white stain (Saharan *et al*., 2016; Cruz and Valent, 2017). Lesions contain gray centers during sporulation, which become white to tan when spores are released (Igarashi *et al*., 1986; Igarashi, 1990). Wheat blast damage to grains varies in severity and timing depending on the infection. The absolute sterility of spikes caused by an infection that occurs before anthesis or at the beginning of blooming leads to seed abortion (Goulart *et al*., 1990; Goulart and Paiva, 1992; Urashima *et al*., 2009). Small, wrinkly, malformed, and low-weight kernels that are in appropriate for human consumption are produced by infection during the grain filling stage (Goulart *et al*., 2007; Malaker *et al*., 2016). Depending on the stage of crop growth in the field, lesions on the leaves may vary in size and shape. Under favorable environmental circumstances, the leaves of particularly susceptible cultivars can get seriously infected at the seedling stage and result in complete plant mortality (Igarashi, 1990; Singh, 2017). At the seedling stage, resistant cultivars may also exhibit a moderately susceptible to susceptible response to the disease (Roy *et al*., unpublished). As the disease progresses, a water-soaked diamond-shaped lesion with a dark brown border turns grayish white in the center on immature seedlings. The entire leaf may wither if many lesions combine (Rios *et al*., 2013). In circumstances that are favorable to MoT in cultivars that are particularly susceptible to it, older leaves are more vulnerable to it than younger ones (Cruz *et al*., 2015). The presence of oval, elongated, or eye-shaped, grey to tan necrotic lesions with dark margins is one of the symptoms on the leaf (Malaker *et al*., 2016). Rarely, lesions can also be detected on the stem, culm, and node of the culm. Elongated or elliptical lesions with a white center and a dark-brown or blackish edge are examples of stem lesions. Due to its resemblance to Fusarium head blight (FHB), caused by

Fusarium graminearum, and spot blotch, caused by *Bipolaris sorokiniana*, respectively, wheat head blast in the field might occasionally be misdiagnosed (Pieck *et al*., 2017; Singh, 2017). Spikelets above the infection point on the rachis may also become bleached from FHB infection, with pink to orange masses of the fungus' spores visible on the infected spikelets as opposed to the gray masses of *MoT* (Wise and Woloshuk, 2010; Valent *et al*., 2016). Dark brown or black staining appears on the infected spikelets in the case of spot blotch, and such spikes may include healthy spikelets at both ends from the infection location. Because of the mixed infection of spot blotch in the field, blast signs on the leaves are frequently difficult to distinguish.

2.5 Epidemiology of wheat blast

There is growing concern that MoT strains could spread to other parts of the world given the prevalence of wheat blast in South America, Bangladesh, and now Zambia. A method for swiftly and accurately detecting the pathogen in questionable samples is necessary for effective surveillance of such a spread (Pieck *et al*., 2017). On questionable samples, wheat blast is typically diagnosed using traditional disease diagnostic techniques, such as visual confirmation of illness signs and the pyriform conidia of the fungus. The wheat blast fungus can infect any sections of the plant that are above ground. However, at the reproductive stage, the most serious infection affects the wheat spikes or peduncles. At the base or lower portions of the rachis, there will first appear to be a black patch or other discoloration. According to Cruz and Valent (2017), an infection in the rachis or peduncle can prevent nutrients from reaching the upper spikelets above the infection spots. The infection areas eventually cause damage to all of the top spikelets, leading to partial or complete bleaching and drying of the spike (although the leaves may still be green). These manifestations are used to identify wheat blast in the field since they are thought to be the disease's most recognizable symptoms. At the site of the rachis infection in highly susceptible cultivars, the fungus can be seen to produce gray, dark-gray, or black spores (Islam *et al*., 2016). Small, shriveled, malformed grains with a lowtest weight are produced in the blast-infected heads, making them unfit for human consumption (Malaker *et al*., 2016). However, infection that takes place prior to anthesis or in the early stages of blooming can completely sterilise spikes, leading to seed abortion (Urashima *et al.*, 2009). In addition to various foliar diseases, particularly spot blotch lesions, elliptical, gray to tan necrotic lesions with dark margins are frequently found on leaves. Additionally, in some areas, the lowest nodes of the stem have a tendency to blacken. The wheat blast-infected plants have green canopies and partially and completely bleached spikes, which are quite similar to the symptoms of Fusarium head blight (FHB), which is brought on by *Fusarium graminearum*. Because of this, FHB is frequently mistaken for the wheat head blast. However, blast-infected spikes typically feature dark-gray sporulation instead of the pink or peach fungal spore masses that are characteristic of FHB-infected spikes (Tembo *et al*., 2020). Additionally, many spikes have the rachis of the fungus visible as grayish mycelium (Malaker *et al*., 2016). When spikes or leaves with blast infection are incubated in moist conditions, 2-septate hyaline pyriform conidia are produced. Due to asymptomatic colonization and the fact that most infections can penetrate tissues that are not visible to the naked eye, disease inspections that rely solely on visual identification of disease symptoms may not be sufficient in the field. In addition, visual evaluations and human evaluations of disease phenotypes in the field necessitate highly qualified professionals. Such processes are frequently laborious and subject to human bias.A portion of the problems with visual disease evaluation may be resolved by recent developments in digital technologies for identifying, diagnosing, and quantifying plant illnesses. In example, recently created sensor-based technologies have made it possible for widespread early plant disease diagnosis (Mahlein, 2016). Sensor-based solutions, however, have not yet been used for early wheat blast detection in the field. Even when virulent MoT strains and susceptible wheat plants are present in the same area, an outbreak of wheat blast is not always certain to spread widely. Through their effects on the host plant and the virus, environmental factors have a significant impact on the epidemic. The availability, growth stage, succulence, and genetic susceptibility of the host plant, as well as the pathogen's survival, vigor, multiplication, sporulation, dissemination, germination, and penetration, are all significantly influenced by moisture and temperature in the environment. Weather patterns that are rainy and humid are favorable for wheat blast outbreaks. When temperatures are between 18 and 25 degrees Celsius during anthesis and there is a period of continuous rain, severe field infections can develop (Kohli *et al*., 2011). According to Cardoso *et al*. (2008), a substantial outbreak of the disease may occur if the ideal temperature was between 25 and 30°C and there was an increase in wetness during a period of 25 to 40 hours.The first hotspots within wheat fields have been identified by several blast researchers and wheat growers in Bolivia and Bangladesh, which could lead to an outbreak of blasts (Islam *et al*., 2020). Additionally, head blast epidemics may occur from MoT sporulation from a very low initial inoculum level before spike initiation if enough secondary inoculum is provided (Cruz *et al*., 2015). According to numerous publications, temperature rise, especially in the winter (Hossain and da Silva, 2013), raises the possibility of wheat blast in Bangladesh. Significant dewfall throughout the winter, which keeps too much moisture on wheat plants for 16–17 hours and encourages fungal sporulation, could be another disease-promoting environmental factor. Significant wheat blast outbreaks have been observed in South America's humid and warm regions, including Bolivia, Paraguay, and northwest Argentina (Kohli *et al*., 2011). High relative humidity, frequent leaf/spike wetness due to persistent rain, and temperatures between 25 and 30°C all work together to promote the growth of wheat blast (Cruz and Valent, 2017; Mottaleb *et al*., 2018). In Bangladesh, the minimum temperature increased noticeably in 2016 across all regions (Islam *et al*., 2019).

2.6 Spread of wheat blast in Bangladesh, South Asia

When a number of reports (Callaway, 2016; Islam *et al.*, 2016; Malaker *et al.*, 2016) confirmed the epidemic presence in eight districts, including Barishal, Bhola, Chuadanga, Jashore, Jhenaidah, Kushtia, Meherpur, and Pabna in the southwestern and southern districts of Bangladesh, it came as a sudden shock and caught the South Asia wheat production regions off guard. Nearly 15,000 hectares (3.5% of Bangladesh's total 0.43 million ha of wheat land) were impacted by this first incident outside of South America, with an average yield loss of 25–30% (Islam *et al.*, 2016; Malaker *et al.*, 2016). Weather conditions during the wheat cropping season were cooler and drier in the next 5 years (2017–2021) and did not encourage WB infection, development, or spread (Mottaleb *et al.*, 2019). Nevertheless, the disease did not only spread to the first eight affected areas, but also to 14 other districts. In 2017, it expanded to four more districts that are adjacent to previously impacted WB districts: Rajshahi, Faridpur, Magura, and Gopalganj. Tangail, Jamalpur, Natore, and Rajbari are four additional new districts where the disease has expanded in 2018. Jamalpur is not near any of the previously impacted districts among them. In 2019, the expansion of WB to the districts of Naogaon, Mymensingh, Madaripur, and Narail is seen. A new district, Bogura, which is located to the nation's northwest and is regarded as the country's primary wheat-producing region, was expected to have WB in 2020 (BWMRI). The disease has now reached Kurigram and Chapainawabganj in 2021, but infection rates there are still very low. The pattern of disease spread made it very evident that Bangladesh is experiencing both seed-borne and air-borne routes of dispersal. According to Mottaleb *et al*. (2018), WB is a threat in a number of Bangladeshi districts that are hotter and more humid.Seven districts, Tangail, Jamalpur, Naogaon, Mymensingh, Kurigram, Chapainawabganj, and Bogura, which are in the northern part of Bangladesh where relatively cooler conditions prevail, have been observed to have WB incidence despite not being predicted as vulnerable to the disease in recent years. The alarming discovery of WB in cooler, drier weather increased South Asia's susceptibility to the disease and demonstrated MoT's capacity to endure adverse circumstances.

2.7 Reactive oxygen species (ROS)

According to Kumar *et al*. (2015) and Parvin *et al.* (2019), reactive oxygen species (ROS) are the primary cause of cell damage under both biotic and abiotic stressors. A considerable number of ROS, including singlet oxygen ($^1O_2$), superoxide (O^{2}-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), are produced during salt stress, according to studies on molecular and biochemical aspects (Hasanuzzaman *et al*. 2019). According to Quiles and Lo'pez (2004) and Mahmud *et al*. (2017), these ROS are highly cytotoxic and are the cause of lipid peroxidation, protein denaturing, DNA mutation, cellular damage, and programmed cell death. According to Mittler *et al*. (2004), oxidative stress depends on the equilibrium between ROS production and ROS scavenging, which in turn depends on development circumstances, the length of the stress, and the tissue's capacity to endure unfavorable circumstances (Costantini 2019). Plants have effective enzymatic and non-enzymatic antioxidant defense systems built into their cells to remove ROS (Bhuyan *et al*. 2020). According to Hasanuzzaman *et al*. (2019), plants also activate non-enzymatic antioxidants like ascorbate (AsA), glutathione (GSH), alkaloids, phenolic compounds, a tocopherol, and nonprotein amino acids in addition to four enzymes of the ascorbate-glutathione cycle. Another extremely cytotoxic and reactive substance created under salinity stress is methylglyoxal (MG). However, the glyoxalase system has two thiol-dependent enzymes called glyoxalase I (Gly I) and glyoxalase II (Gly II) that can detoxify MG (Hasanuzzaman *et al*. 2017). Exogenous application of phytoprotectants, such as signaling molecules, osmolytes, plant hormones, trace elements, etc., is one of the most widely used techniques to improve abiotic stress tolerance (Hasanuzzaman *et al*. 2018). Fungicides' impact on plant infections has already been researched, but as our understanding of plant signaling cascades has grown, some fungicides, when used in small concentrations, have demonstrated cellular defenses like antioxidant defense.Triazole and strobilurin fungicides have increased antioxidant activity and alkaloid production in plants, as well as distinct physiological and morphological variance (Ruske *et al.*, 2003, 2004; Zhang *et al.*, 2010). Triazole and strobilurin fungicides have been reported to boost the salt-tolerance mechanisms in plants, according to research by Nabati *et al*. (1994) and Filippou *et al*. (2016). The fungicides triazole and strobilurin may reduce salt-induced damage by preserving ion homeostasis, although their effects on the antioxidant and glyoxalase systems' defensive mechanisms have not yet been thoroughly investigated. Salinity can have a significant impact on the growth and output of wheat (*Triticum aestivum* L.), a significant cereal crop (Chaves *et al.*, 2013). However, there aren't many research that have concentrated on the methylglyoxal (MG) detoxification pathway and ROS metabolism in plants when fungicides are applied exogenously. In order to better understand how tebuconazole (TEB, triazole fungicide) and trifloxystrobin (TRI, strobilurin fungicide) affect wheat seedlings' physiology, oxidative/antioxidative status, and MG detoxifying system.

2.8 ROS in disease resistance

Plant resistance to disease attack and co-evolutionary tactics have always been incremental and crucial for agricultural production systems over time. The recognition and signaling mechanisms between the host and the pathogen regulate plant susceptibility and resistance to external invasion by diseases. A signaling cascade that produces ROS, phytoalexins, and anti-microbial genes is activated bymicrobe/pathogen-associated molecular patterns, or MAMPs/ PAMPs, and Avr (avirulent) gene products that correlate to their host receptors (plants). This also triggers a variety of plant defense genes that are effective against a variety of diseases.This also turns on a variety of plant defense genes that are effective against a variety of diseases. According to Goraya *et al.* (2016), plants produce ROS through the electron transport chain in their mitochondria and chloroplasts as well as through peroxisomal photorespiration. The formation of apoplastic ROS in the plasma membrane is mediated by

RBOH (respiratory burst oxidase homolog) genes for NADPH oxidase, polyamine oxidase (PAO)-mediated destruction of spermidine, and oxalate oxidase, H_2O_2 (hydrogen peroxide), superoxide (O^{2-}), (hydroxyl) (•OH), and singlet oxygen ($^1O_2$) are the most stable and prevalent ROS in plants.Between the four, there is a quick interconversion that offers more functional versatility. H_2O_2 is the most stable of the four classes and is carried by ROS through aquaporin membranes (Foyer *et al*. 2018). differing physiological effects are caused by differing ROS generation concentrations. ROS can act as a signaling agent in low doses. However, the excessive buildup of ROS may result in cell death due to its detrimental oxidative effects on lipids, proteins, and nucleic acids. A ROS wave is produced by the interaction of calcium channels, NADPH oxidases (NOX), and calcium fluxes brought on by oxidative stress and is capable of transducing long-distance signals (Smirnoff *et al*., 2018).An ROS wave is a cell-to-cell self-replicating mechanism of ROS production that is mediated by respiratory burst-homolog-D (RBOHD). Once activated, it causes a single cell to produce more ROS than usual, acting as a sensory signal to neighboring cells to also produce more ROS. There is coordination between various generated stressors and the whole-plant systemic stomatal response in a recently described unique role of the ROS wave (Smirnoff *et al*., 2018). According to Jan *et al*. (2018), the ROS wave is a cell-to-cell auto-propagating mechanism of ROS production that is driven by the protein known as respiratory burst homolog D (RBOHD). It causes the cell to produce more ROS when it is activated in a single cell. As a result, ROS buildup at the apoplast (RBOHD generates ROS at the apoplast) occurs (Mittler *et al*., 2011).

2.9 Defense system against ros production and accumulation

At the turn of the century, scientists learned about the ROS defense mechanism in plants and its numerous functions. Recently, it was determined that the defense system against ROS includes both enzymatic and non-enzymatic defensive mechanisms, and that these defense mechanisms are triggered by various environmental pressures from the outside world (Noctor *et al*., 2018).

Various enzymatic and non-enzymatic defense mechanisms control the formation of ROS, which is a byproduct of routine cellular metabolism (Huang *et al.*, 2019). According to Karpinska *et al*. (2001), non-enzymatic defense mechanisms include glutathione (GSH), ascorbic acid (AA), phenolic compounds, and tocopherols (TOCs), while enzymatic defense mechanisms include APX, CAT, SOD, and GPX. Plants have numerous ROS defense mechanisms that include both enzymatic and non-enzymatic components. In diverse cell components, such as peroxisomes, chloroplasts, and mitochondria, ROS production and scavenging can occur. In the case of such pathways, there is substantial coordination between these organelles (Pang *et al*., 2008).In plants, the production and scavenging of ROS are in equilibrium under normal circumstances, but under stress, this equilibrium is upset, causing an increase in ROS levels (Pang *et al*. 2008), which causes oxidative stress to cell components. However, in more advanced plants, there is a natural defense system to counteract this increase in ROS levels (Sharma *et al*. 2019, 2019). To lower ROS levels in plants during abiotic stressors, the ROS defense mechanism is crucial. Plants have developed sophisticated defense mechanisms over time to combat the buildup and generation of ROS (Berni *et al*., 2019).

2.10 Tebuconazole fungicide against plant stress

Triazole fungicides are frequently used to treat a variety of fungal diseases in cereals, vegetables, fruits, and teas because they have a broad antifungal action (de Albuquerque, Carrao, Habenschus, & de Oliveira, 2018). In order to control fungal diseases in crops, a class of extremely effective agricultural fungicides known as triazole pesticides is frequently utilized (Bakanov *et al*., 2020). There are many fungicides belong to this group such as Tebuconazole, Hexaconazole, Difenoconazole, Myclobutanil etc. Due to its high absorbability and durability, tebuconazole (TEB) is typically sprayed on vegetables, cereals, fruits, tobacco, and noncrops (Kundu *et al*., 2014). Tebuconazole, a typical triazole fungicide, is currently one of the most popular fungicides in the world. Its attributes of great effectiveness, broad spectrum, minimal residue, lengthy duration, and strong absorption have led to its widespread use. When used at the recommended amount, the triazole fungicide tebuconazole may have an impact on the growth, symbiosis, grain yield, and nutrient uptake of pea plants (Ahemad and Khan, 2011). When tebuconazole was given excessively during leaf or seed treatment for maize, it will result in phytotoxicity and slow down plant growth (Yang *et al*., 2016). It was also possible to examine alterations in plant metabolism at the protein and DNA levels in addition to metabolic reactions. After seed treatment with several fungicides, soluble proteins for cotton plants were shown to be drastically reduced (Mohamed and Akladious, 2017). Comet assay tests on the roots and meristematic cells of *Lactuca sativa* revealed that tebuconazole had phytotoxic, cytotoxic, and genotoxic effects (Arago *et al*., 2021). To increase the resilience of tebuconazole-coated maize seeds to chilling stress, the expression of gibberellin regulatory genes was controlled (Yang *et al*., 2016).

2.11 Fungicides for wheat blast management

Given the limited impacts of varietal resistance, fungicides are currently essential for WB management. A susceptible variety can be used to determine a fungicide's effectiveness, however the results are not very encouraging and were found to depend on the cultivar in South America while being successful in Bangladesh (Kohli *et al*., 2011; Roy *et al*., 2020). Fungicides lose their efficacy when the disease pressure is high or the environment is hospitable (Kohli *et al.*, 2020). According to reports, in some regions of Brazil, even four sprays were occasionally insufficient to entirely eliminate WB infection (Urashima *et al*., 2017), proving the critical necessity for genetic resistance used in conjunction with fungicides (Ceresini *et al.*, 2018).Fungicide administration at the seedling stage is also crucial to lowering inoculum load on basal or older leaves, even though this stage is when they are most frequently utilized (Cruz *et al*., 2015). Wheat blast has been combated with foliar and seed fungicide applications, either alone or in combination. According to Buerstmayr *et al*. (2017), fungus can continue to develop on cotyledons and main leaves while infected seeds are germination. As a result, it is advised to treat seeds with fungicides like benomyl (Sadat and Choi, 2017), difenoconazole (Yesmin *et al*., 2020), and carboxin + thiram (BWMRI, 2020). In Brazil and Bolivia, respectively, a mixture of QoI + DMI (quinone outside inhibitor, QoI, and demethylation inhibitors, DMI) and a spray of fungicides based on mancozeb were found to be effective (Cruz *et al.*, 2019).Farmers in Bangladesh are also encouraged to use a combination of triazole and strobilurin fungicides, such as Nativo 75 WG and Amister Top 325 SC (Sadat and Choi, 2017; BWMRI, 2020; Roy *et al*., 2020a). MoT isolates found in Bangladeshi farmer fields showed that carbendazim (Autostin 50WGD, Knowin 50WP), and QoI + DMI fungicides, namely Nativo 75WG (tebuconazole + trifloxistrobin), were totally able to inhibit MoT mycelial growth in in vitro experiments. However, according to Debnath *et al*. (2019), the two mancozeb-based fungicides applied in the same trial were ineffective. The fact that DMI + QoI fungicides are effective in Bolivia but not in Brazil suggests that the two nations' prevalent MoT isolates and environmental circumstances are different.In Brazil, strobilurin fungicides, which are of the QoI type and target the pathogen's mitochondrial respiration, were widely used to combat the disease. New MoT isolates with altered mitochondrial cytb genes have appeared in recent years Castroagudin *et al*. (2015), Singh *et al*. The prevalence of this mutation has increased from 36 to 90% in the sampled population over a 7-year period (2005–2012) (Castroagudin *et al*., 2015).A novel class of fungicide known as SDHI (succinate dehydrogenase inhibitors) has seen increased use recently. It is advised to use SDHI fungicides in combination or in rotation with other kinds of fungicides because it is quite possible that *MoT* would develop resistance to them if they are used alone (Ceresini *et al*., 2019).

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in net house of Department of Plant Pathology, and Laboratory of Stress Physiology and Molecular Biology, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.

3.2 Experimental period

The experiment was conducted during the period of July 2022 to June 2023.

3.3 Experimental design

The experiment was designed following a Completely Randomized Design (CRD) with three replications.

3.4 Collection of pathogen

The fungus *Magnaporthe oryzae triticum* (MoT) was collected from the Laboratory of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207. After collection, potato dextrose agar (PDA) media was used to preserve the fungus (Fig. 1). For inoculation of MoT in wheat plants, spore suspension was adjusted 2×10^4 conidia mL^{-1} counted by hemocytometer described by Mohsin *et al*. (2016).

3.5 Collection of plant materials

Wheat seeds (BARI Gom 26 and BARI Gom 33) were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

Fig. 1 *Magnaporthe oryzae triticum* under compound microscope

3.6 Growing condition of wheat plants and application of treatments

Wheat (*Triticum aestivum* L. cv. BARI Gom 26 and BARI Gom 33) seeds were grown hydroponically. The seeds were sterilized (by 70% ethanol) and soaked in distilled water (DH_2O) for 4 h. After soaking, the seeds were sown in plastic pots (8 cm diameter, a volume of 250 mL) hydroponically and placed in the net house. During the growing period, the seedlings were nourished with half-strength Hoagland solution as a nutrient. The nutrient solution was renewed after every 5 days. At 10 days, the seedlings were inoculated with the spore suspension of MoT by spraying and covered with plastic pot for maintaining proper humidity. After 03 days of inoculation, recommended dose (0.3 g L^{-1}) of Teboconazole fungicide was sprayed. Therefore, six treatments were used to couduct the experiment. Such as- i. BARI Gom 26 (V1), ii. BARI Gom 26 + MoT (V1P), iii. BARI Gom 26 + MoT + Tebuconazole (V1PF), iv. BARI Gom 33 (V2), v. BARI Gom 33 + MoT (V2P), vi. BARI Gom 33 + MoT + Tebuconazole (V2PF). After the appearance of disease symptom, sixteen-day-old seedlings were used to observe physiological and biochemical attributes (Fig. 2).

3.7 Estimation of number of infected plants pot^{-1}

In naked eye, the numbers of infected plants are counted and then estimated the average number of infected plants per pot.

3.8 Estimation of number of lesion plant^{-1}

The number of lesion per plant was observed the estimated the average number of lesion per plant.

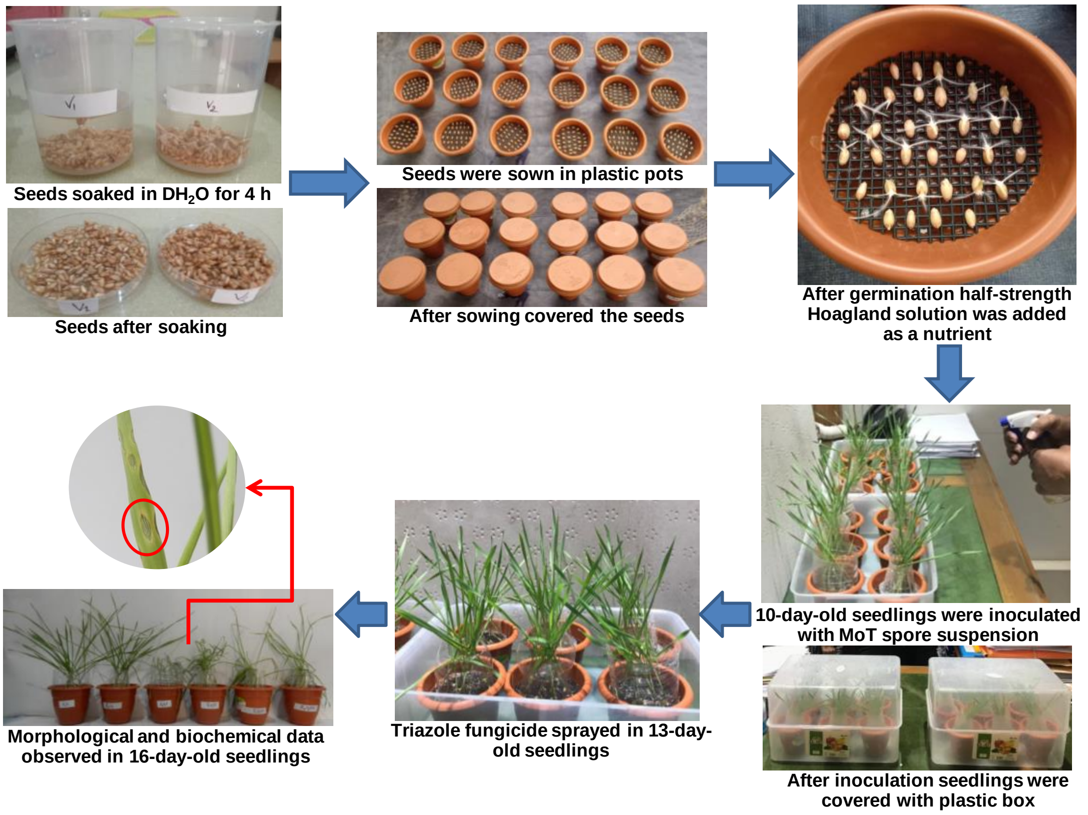

Fig. 2 Diagrammatically represent the growing conditions and treatment application in wheat seedlings

3.9 Measurement of malondialdehyde content

The lipid peroxidation level was measured in terms of malondialdehyde (MDA) content following the method of Heath and Packer (1968). The fresh leaves were extracted using trichloroacetic acid (TCA) and centrifuged at 11,500×g. The collected supernatant was mixed with thiobarbituric acid (TBA) reagent, and incubated in a hot water bath for 30 min. Thereafter, the mixture was quickly cooled to stop the reaction, and then the optical absorbance was recorded at 532 nm and corrected at 600 nm (Fig. 3). Malondialdehyde content was measured using the following formula—

MDA = (Absorbance×reaction mixture×extration mixture)/ (Plant sample×extinction coefficient)×1000 (n mol/gFW)

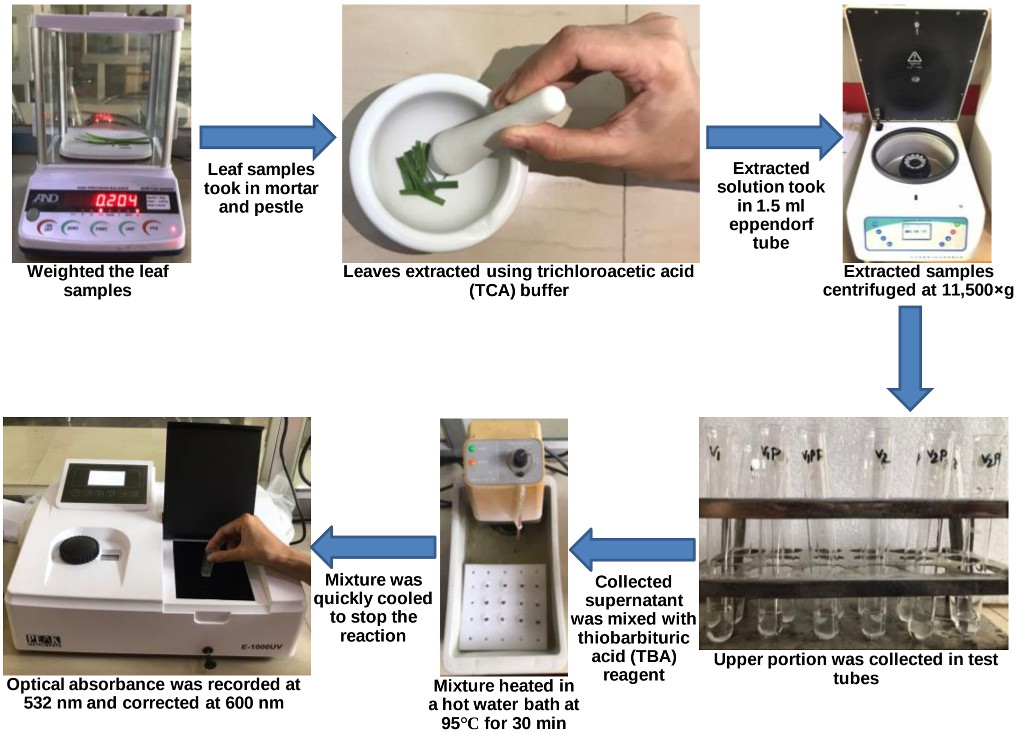

Fig. 3 Diagrammatically represent the measurement of malondialdehyde content

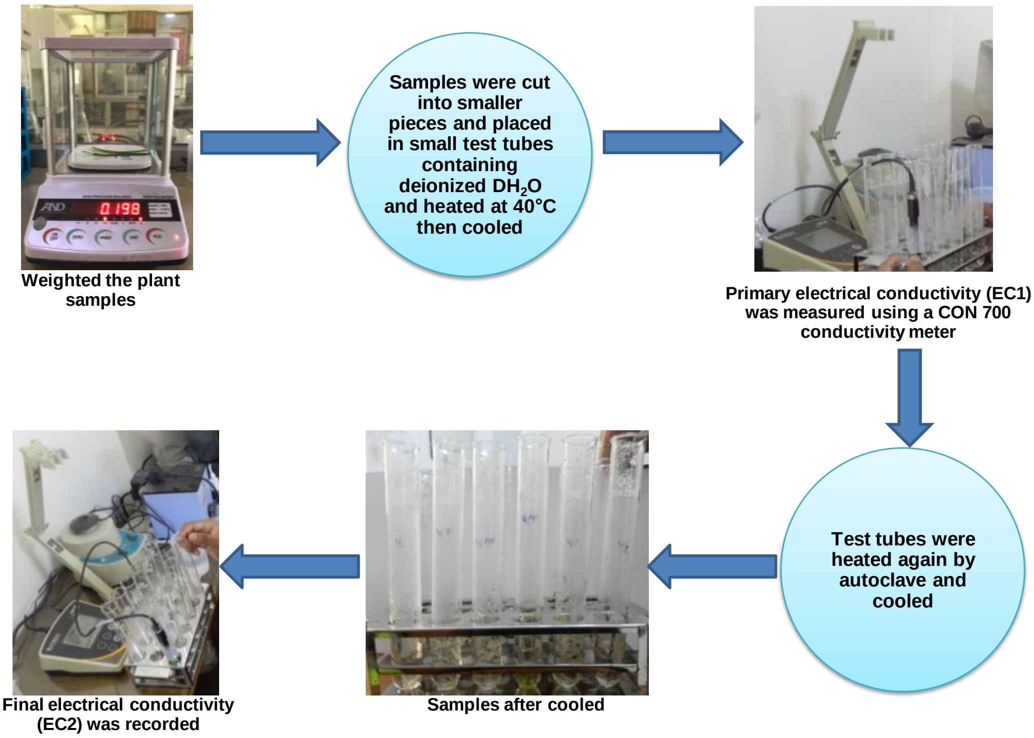

Fig. 4 Diagrammatically represent the measurement of shoot and root electrolyte leakage

3.10 Observation of shoot and root electrolyte leakage

Electrolytic leakage (EL) was observed following the method of Dionisio-Sese and Tobita (1998). Smaller pieces of leaf tissue were put into test tubes containing deionized DH_2O and heated at 40°C for 1 h. The test tubes were then cooled at room temperature and primary electrical conductivity (EC_1) was measured using a CON 700 conductivity meter (Eutech Instruments, Singapore). The test tubes were heated again by autoclave and after cooling, the final electrical conductivity (EC_2) was recorded (Fig. 4). Electrical Leakage were measured using the following formula—

Electrical Leakage (EL, %) = $EC_1/ EC_2 \times 100$.

3.11 Histochemical detection of hydrogen peroxide and super oxide generation

To detect hydrogen peroxide (H_2O_2) and super oxide ($O_2^{\bullet-}$) generation, 0.01% of acidic 3,3′-diaminobenzidine (DAB) and nitroblue tetrazolium chloride (NBT) solution were used, respectively (Chen e*t al*. 2010). The leaves were immersed in DAB and NBT solution separately for 24h and incubated at 25°C. Therefore, the leaves were washed in 70% ethanol followed by DH_2O to remove DAB and NBT solution. Afterward, the leaves were heated at 70°C until clear brown and blue spots were seen, which is the indicator of H_2O_2 and $O_2^{\bullet-}$ generation, respectively.

3.12 Determination of relative water content

Relative water content (RWC) in the leaves was determined according to Barrs and Weatherley (1962). Leaf FW, DW, and turgid weight (TW) were determined and RWC was then calculated by following formula: RWC (%) = [(FW-DW)/(TW-DW)]×100.

3.13 Determination of photosynthetic pigment content

Photosynthetic pigment (chlorophyll, Chl and carotenoid, Car) was measured according to Lichtenthaler (1987). Leaf samples (0.1 g) were cut into smaller pieces and placed in small test tubes containing 100% ethanol. The samples were heated in a hot water bath for 30 min at 60°C, and then cooled. Absorbance was measured spectrophotometrically at 664, 648, and 470 nm (Fig. 5). The content of Chl *a*, Chl *b* and carotenoid were measured using the following formula—

Chl $a = 0.0127 \times \text{Abs}_{(664)} - 0.00269 \times \text{Abs}_{(648)} \times 10/0.1$ [10 ml ethanole, 0.1 g plant tissue]

Chl $b = 0.0229 \times \text{Abs}_{(648)} - 0.00468 \times \text{Abs}_{(664)} \times 10/0.1$ [10 ml ethanole, 0.1 g plant tissue]

Carotenoid= $\{1000 \times \text{Abs}_{(470)}\} - (3.27 \times \text{Chl } a) - (104 \times \text{Chl } b) / (198 \times 1000 \times 10 \times 0.1)$

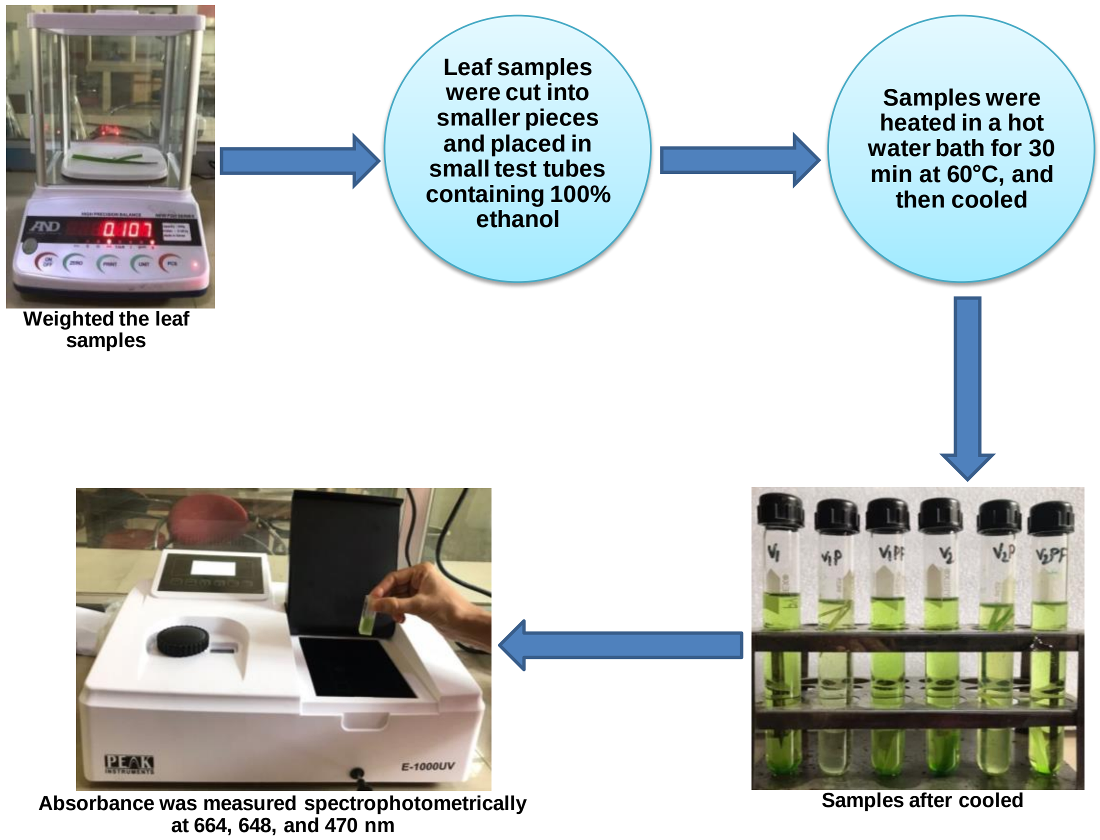

Fig. 5 Diagrammatically represent the determination of photosynthetic pigment content

3.14 Measurement of shoot and root length

Shoot and root length (cm) of wheat seedlings were measured using a measuring scale then computed the average shoot and root length of wheat seedlings.

3.15 Determination of shoot and root fresh and dry weight

After harvest, the fresh weight (FW) of the shoots and roots were determined immediately by using precision balance, and the dry weight (DW) was measured after drying both shoot and root at 70°C for 48 h.

3.16 Statistical analysis

All the recorded data were analyzed using R software (version 4.1.3) by subjecting them to one-way analysis of variance (ANOVA). The means were compared using Fisher's Least Significant Difference (LSD) test, where $P \leq 0.05$ was considered significant.

CHAPTER IV

RESULTS

4.1 Phenotypic appearance of wheat seedlings

The inoculation of MoT spore suspension deteriorated the growth of the wheat seedlings (Fig. 6). The seedlings treated with MoT resulted in typical blast symptoms observed on the leaves and then most of the leaves were withering. Exogenous application of triazole fungicide improved the phenotypic appearance of the wheat seedlings by reducing the infection of blast disease.

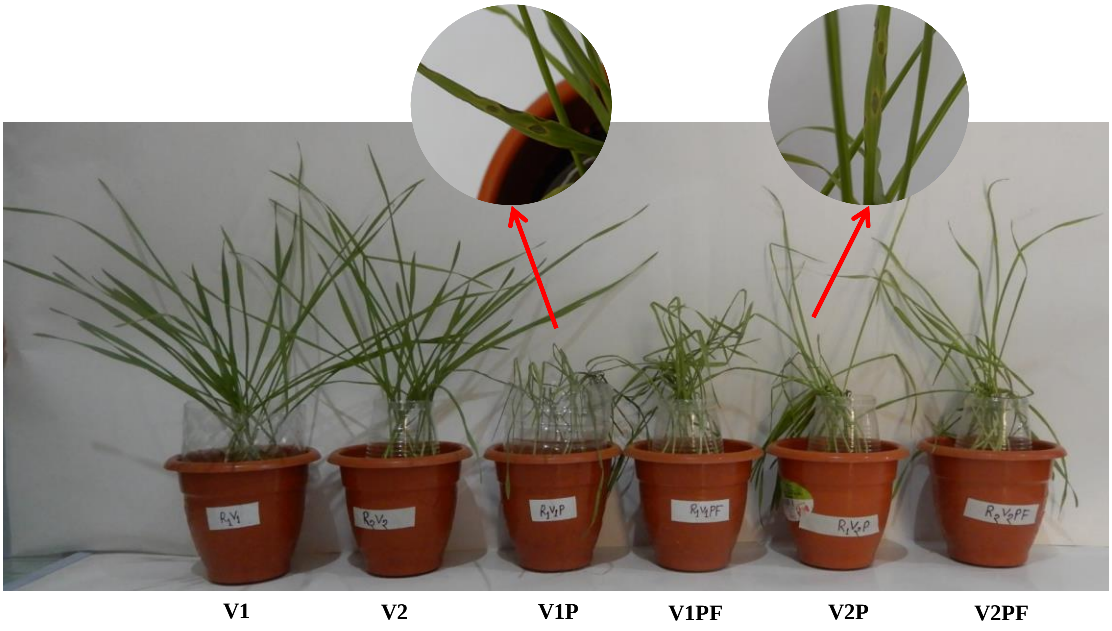

Fig. 6 *In vitro* evaluationof Tebuconazole (0.3 g L^{-1}) on visual difference in sixteen-day-old hydrophonically grown wheat seedlings under blast disease condition, for six days. Here, V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3g L^{-1} Tebuconazole, respectively.

4.2 Number of infected plants pot^{-1}

The inoculation of MoT large number of plants infected and showed blast symptom on the leaves. The number of infected plants pot^{-1} in BARI Gom 33 (V2P) was lower (32.14%) compared to BARI Gom 26 (V1P). After application of fungicide, number of infected plants pot^{-1}reduced in both wheat varieties. Fungicide application reduced 24.32% infected plant pot^{-1} in BARI Gom 26 and 75% in BARI Gom 33 compared to respective MoT treated plants only (Table 1).

Table 1 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on number of infected plants pot^{-1} and number of lesions plant^{-1} in sixteen-day-old hydrophonically grown wheat seedlings under blast disease condition, for six days. Means (±SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test.

Treatments	Number of infected plants pot^{-1}	Number of lesions plant^{-1}
V1	0.00±0 d	0.00±0 d
V1P	12.33±1.15 a	7.67±1.15 a
V1PF	9.33±1.15 b	5.67±0.57 b
V2	0.00±0 d	0.00±0 d
V2P	9.33±0.57 b	6.67±1.15 ab
V2PF	5.33±0.57 c	3.33±0.57 c
CV (%)	12.31	19.17
LSD value	1.33	1.32

Here, V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

4.3 Number of lesions plant^{-1}

The number of lesion plants^{-1} in BARI Gom 33 (V2P) was lower (13.04%) compared to BARI Gom 26 (V1P). After application of fungicide, number of lesion plants^{-1} reduced in both wheat varieties. Fungicide application reduced at 26.09% infected plant pot^{-1} in BARI Gom 26 and 50% in BARI Gom 33 compared to respective MoT treated plants only (Table 1).

4.4 Malondialdehyde content

The seedlings exposed to blast disease produced higher MDA content. Higher MDA content was observed in BARI Gom 26 (V1P) compared to BARI Gom

33 (V2P) (Fig. 7). In BARI Gom 26, MDA content increased by 102.80% in blast disease infected plant compared to control plant. The application of fungicide, MDA content reduced by 17.99% compared to blast infected plant only. Similarly, in BARI Gom 33, MDA content increased by 163.96% in blast disease infected plant compared to control plant. The application of fungicide, MDA content reduced by 9.22% compared to blast infected plant only.

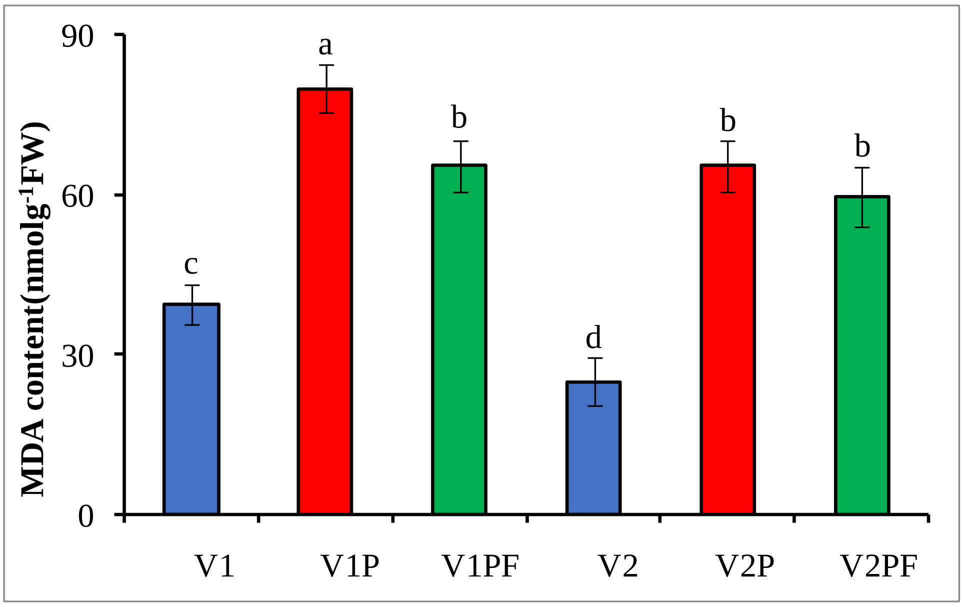

Fig. 7 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on MDA content of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

4.5 Shoot and root electrolyte leakage

The infection of blast disease increased both shoot and root electrolyte leakage (Fig. 8A, 8B). In BARI Gom 26, shoot and root electrolyte leakage increased by 85.28 and 48.53%, respectively in blast disease infected plant compared to control plant. The application of fungicide, shoot and root electrolyte leakage reduced by 24.36 and 24.89%, respectively compared to blast infected plant only. Similarly, in BARI Gom 33, shoot and root electrolyte leakage increased by 304.12 and 112.70%, respectively in blast disease infected plant compared to control plant. The application of fungicide, shoot and root electrolyte leakage reduced by 29.56 and 11.91%, respectively compared to blast infected plant only.

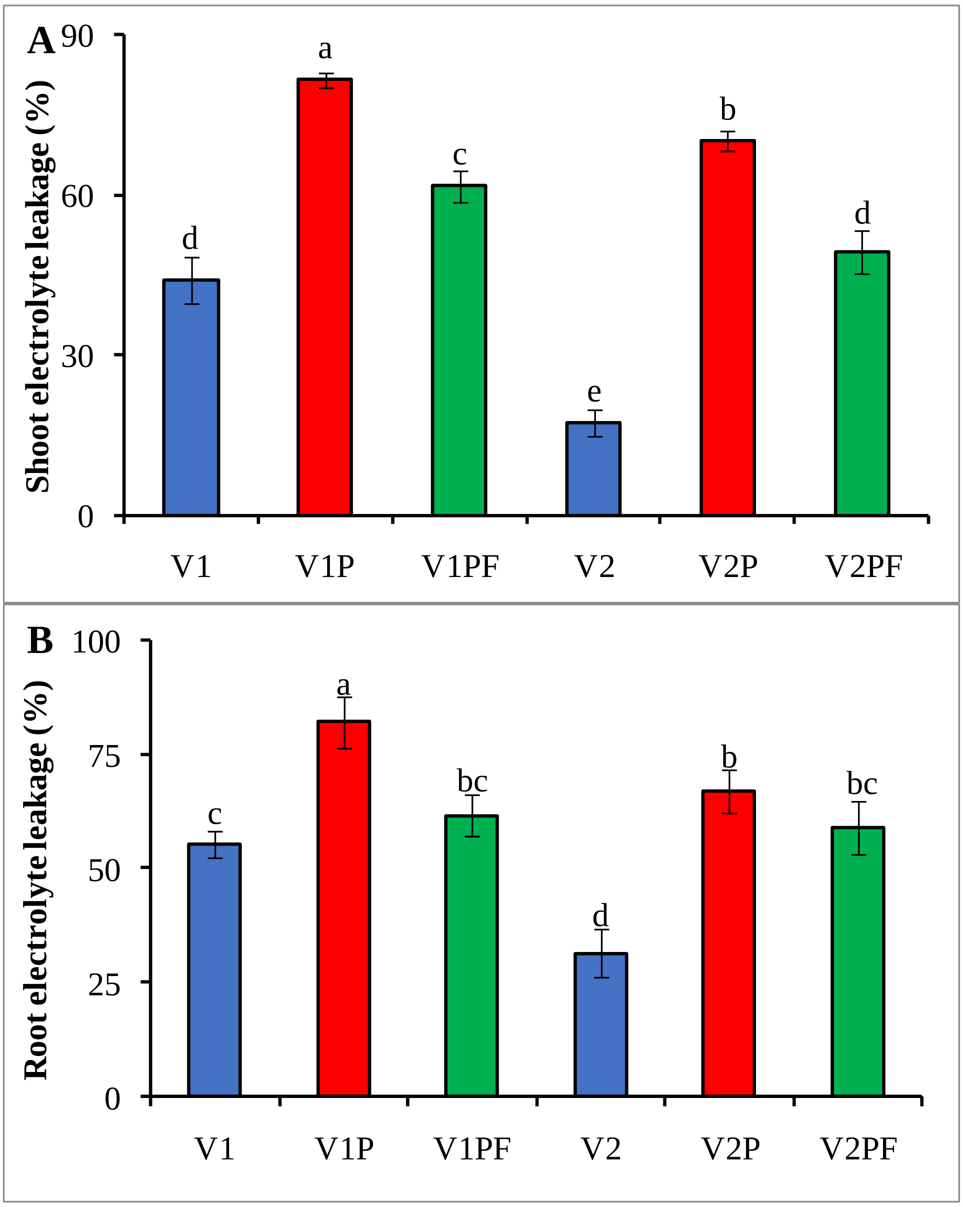

Fig. 8 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on shoot (A) and root (B) electrolyte leakage (%) of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

4.6 Histochemical detection of hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$)

The production of H_2O_2 (brown spots) and $O_2^{\bullet-}$ (blue spots) in the leaves of the blast-treated wheat seedlings were revealed through histochemical detection. Fewer brown and blue spots in the fungicide-treated seedlings indicated reduced production of H_2O_2 and $O_2^{\bullet-}$, compared with the pathogen alone-treated seedlings (Fig. 9).

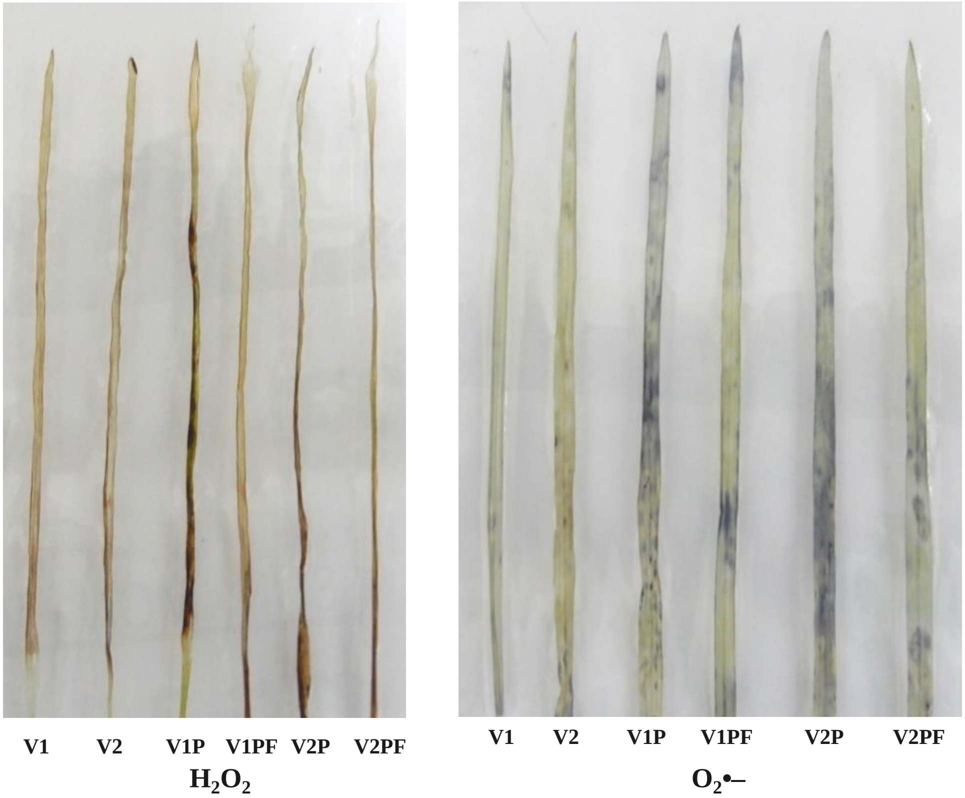

Fig. 9 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on histochemical detection of H_2O_2 and $O_2^{\bullet-}$ of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

4.7 Leaf relative water content

The inoculation of MoT relative water content decreased in both wheat varieties (Fig. 10). The relative water content in BARI Gom 33 (V2) was higher (7.53%) compared to BARI Gom 26 (V1). The infection of blast disease, relative water content decreased by 50.18 and 51.26% in BARI Gom 26 and BARI Gom 33, respectively compared to control plant. After application of fungicide, relative water content improved by 32.28 and 29.03% in BARI Gom 26 and BARI Gom 33, respectively compared to diseased plant.

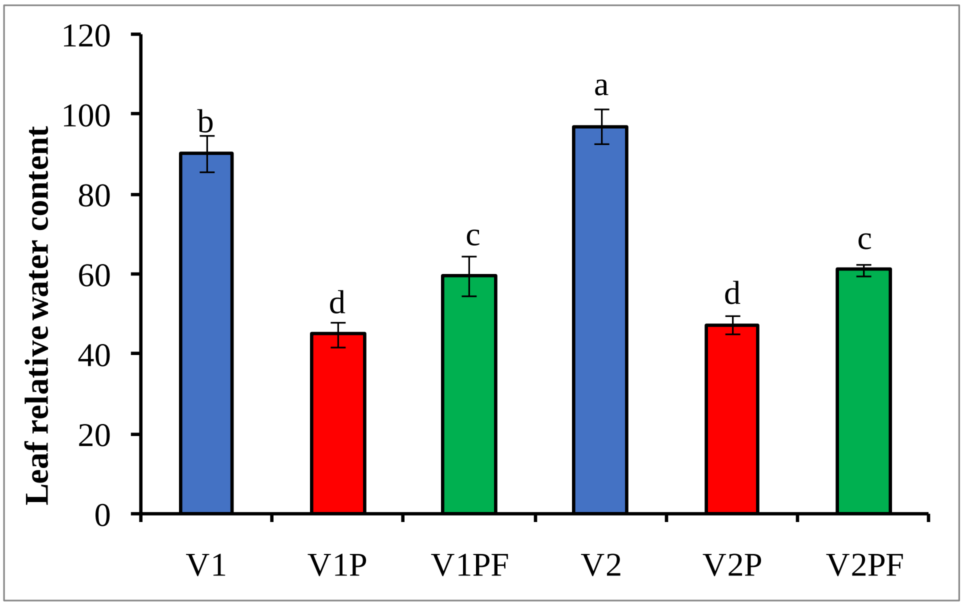

Fig. 10 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on leaf relative water content of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

4.8 Photosynthetic pigment content

The inoculation of MoT photosynthetic pigment content decreased in both wheat varieties (Fig. 11A−D). The Chl *a* content in BARI Gom 33 (V2) was higher (39.70%) compared to BARI Gom 26 (V1). The infection of blast disease, Chl *a* content decreased by 45.25 and 53.60% in BARI Gom 26, BARI Gom 33, respectively compared to control plant. After application of fungicide, Chl *a* content improved by 18.42 and 30.18% in BARI Gom 26 and BARI Gom 33, respectively compared to diseased plant (Fig. 11A).

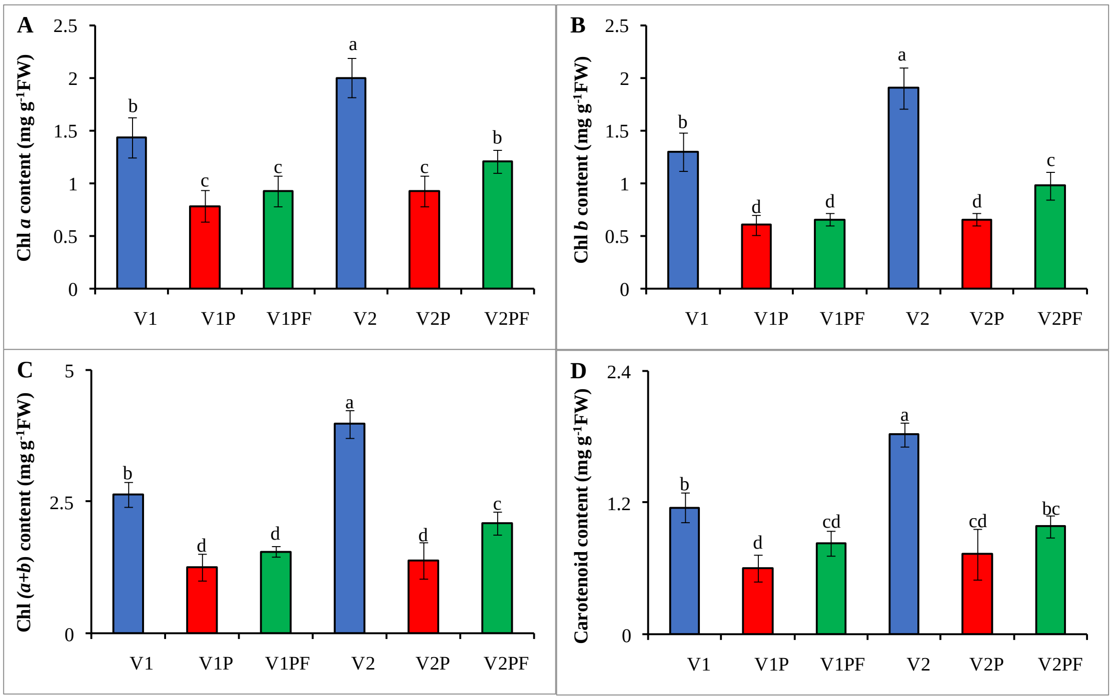

Fig. 11 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on Chl *a* (A), Chl *b* (B), Chl *(a+b)* (C), carotenoid (D) of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

The Chl *b* content in BARI Gom 33 (V2) was higher (46.45%) compared to BARI Gom 26 (V1). The infection of blast disease, Chl *b* content decreased by 53.49 and 65.45% in BARI Gom 26 and BARI Gom 33, respectively compared to control plant. After application of fungicide, Chl *b* content improved by 8.79 and 48.78% in BARI Gom 26 and BARI Gom 33, respectively compared to diseased plant (Fig. 11B).

The Chl *(a+b)* content in BARI Gom 33 (V2) was higher (50.87%) compared to BARI Gom 26 (V1). The infection of blast disease, Chl *(a+b)* content decreased by 52.30 and 65.09% in BARI Gom 26 and BARI Gom 33, respectively compared to control plant. After application of fungicide, Chl *(a+b)* content improved by 23.69 and 50.56% in BARI Gom 26 and BARI Gom 33, respectively compared to diseased plant (Fig. 11C).

The carotenoid content in BARI Gom 33 (V2) was higher (57.76%) compared to BARI Gom 26 (V1). The infection of blast disease, carotenoid content decreased by 47.62 and 59.87% in BARI Gom 26 and BARI Gom 33, respectively compared to control plant. After application of fungicide, carotenoid content improved by 37.39 and 34.45% in BARI Gom 26 and BARI Gom 33, respectively compared to diseased plant (Fig. 11D).

4.9 Shoot and root length (cm)

The infection of blast disease decreased both shoots and root length (Fig. 12A, 12B). In BARI Gom 26, shoot and root length decreased by 36.30 and 17.75%, respectively in blast disease infected plant compared to control plant. The application of fungicide, shoot and root length improved by 22.14 and 13.69%, respectively compared to blast infected plant only. Similarly, in BARI Gom 33, shoot and root length decreased by 31.61 and 28.80%, respectively in blast disease infected plant compared to control plant. The application of fungicide, shoot and root length improved by 21.23 and 27.56%, respectively compared to blast infected plant only.

4.10 Shoot fresh and dry weight (g)

The infection of blast disease decreased both shoots fresh and dry weight (Fig. 13A, 13B). In BARI Gom 26, shoots fresh and dry weight decreased by 74.96 and 44.74%, respectively in blast disease infected plant compared to control plant. The application of fungicide, shoots fresh and dry weight improved by 156.16 and 30.69%, respectively compared to blast infected plant only. Similarly, In BARI Gom 33, shoots fresh and dry weight decreased by 59.13 and 32.76%, respectively in blast disease infected plant compared to control plant. The application of fungicide, shoots fresh and dry weight improved by 73.63 and 18.80%, respectively compared to blast infected plant only.

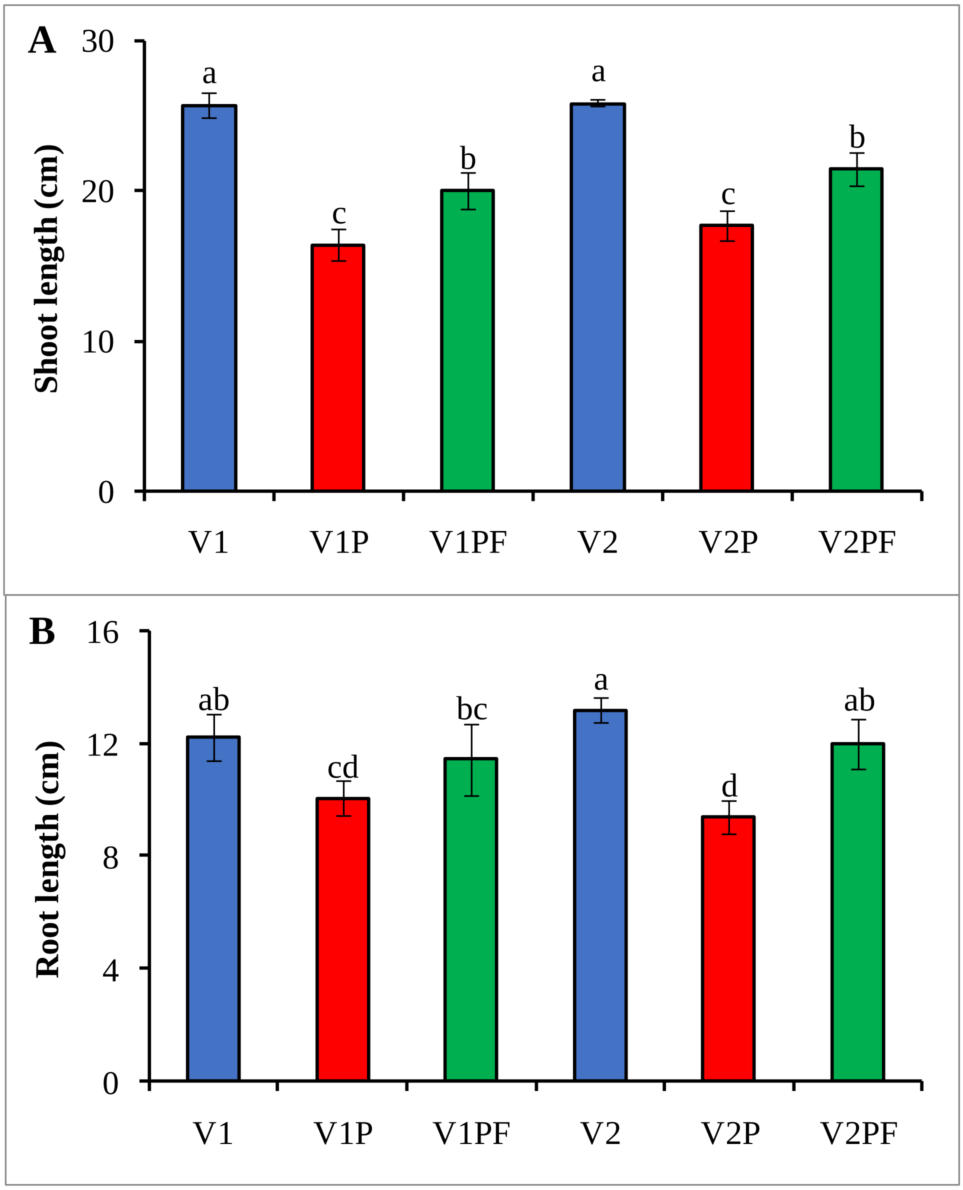

Fig. 12 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on shoot (A) and root (B) length (cm) of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

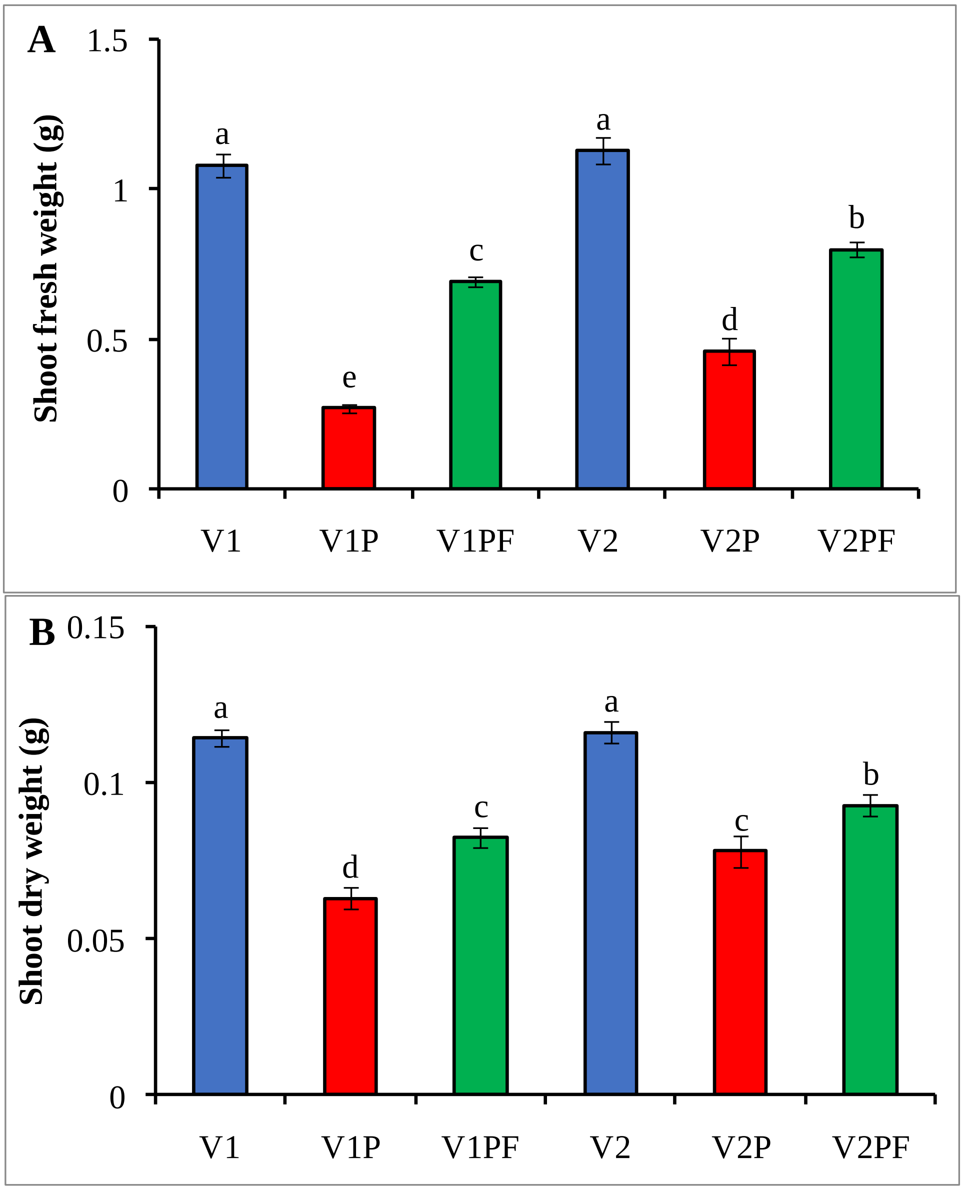

Fig. 13 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on shoot fresh (A) and dry (B) weight (g) of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

4.11 Root fresh and dry weight (g)

The infection of blast disease decreased both root fresh and dry weight (Fig. 14A, 14B). In BARI Gom 26, root fresh and dry weight decreased by 43.51 and 56.60%, respectively in blast disease infected plant compared to control plant.

The application of fungicide, root fresh and dry weight improved by 39.53 and 15.22%, respectively compared to blast infected plant only. Similarly, in BARI Gom 33, root fresh and dry weight decreased by 36.66 and 41.07%, respectively in blast disease infected plant compared to control plant. The application of fungicide, root fresh and dry weight improved by 38.97 and 12.12%, respectively compared to blast infected plant only.

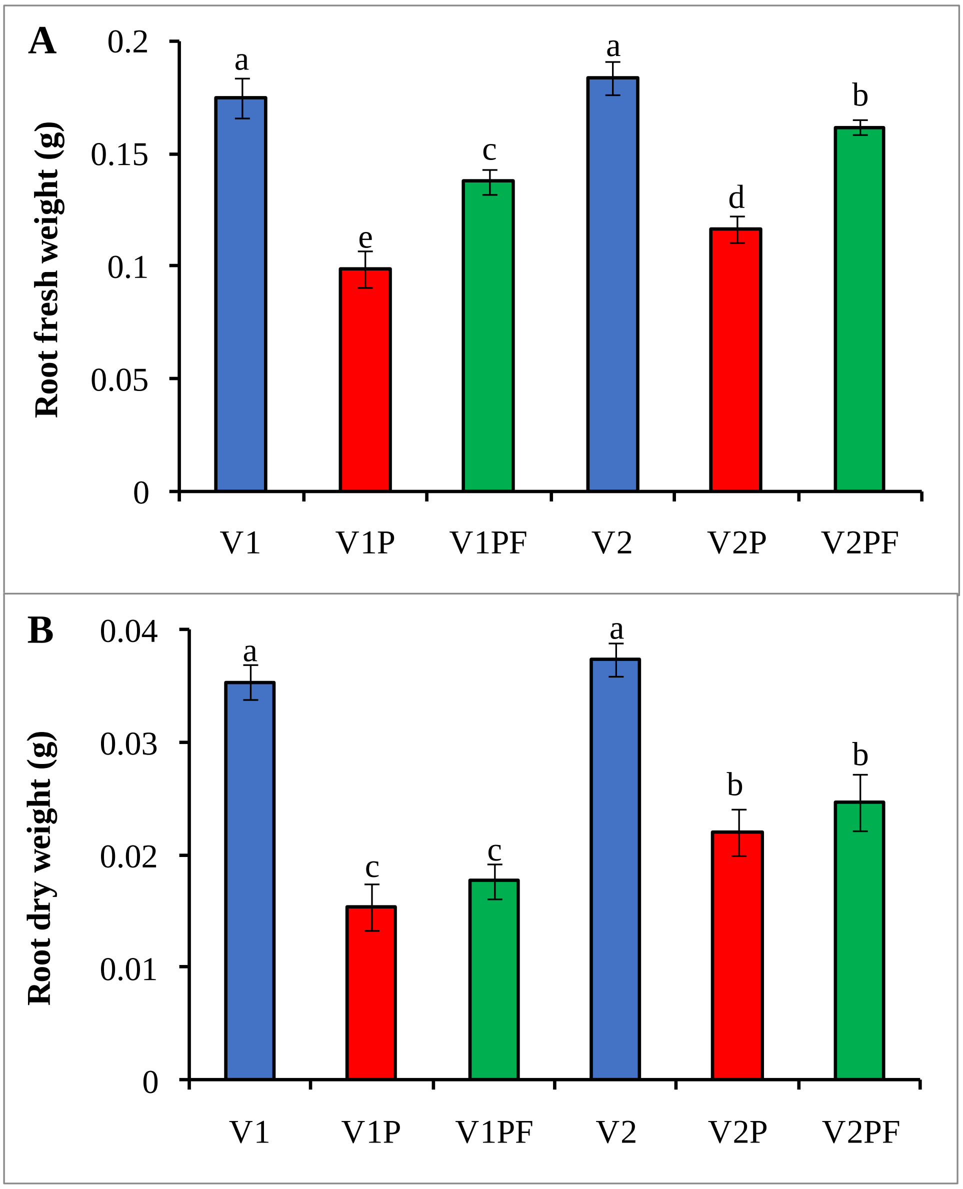

Fig. 14 *In vitro* evaluation of Tebuconazole (0.3 g L^{-1}) on root fresh (A) and dry (B) weight (g) of sixteen-day-old hydroponically grown wheat seedlings under blast disease condition, for six days. V1, V2, P, and F indicate BARI Gom 26, BARI Gom 33, *Magnaporthe oryzae triticum*, and 0.3 g L^{-1} Tebuconazole, respectively.

CHAPTER V

DISCUSSION

Wheat blast is considered a major disease affecting wheat production. This disease can reduce both yield and grain quality of wheat (Goulart *et al*. 2007). When this disease affect the wheat seedling, it hampered the overall growth of the seedling such as decreased plant height, small leaves, lower the number of leaves etc. BARI Gom 26 (V1) is more suceptable than BARI Gom 33 (V2). For that reason, the growth of the V1 variety is decreased a lot due to the infection of MoT than the V2 variety. Similar observation was made by Goulart *et al*. (2007) that the highly susceptible cultivars produced grains from blast-infected spikes are often small, shriveled and deformed, with low test weight (Goulart *et al*. 2007). Due to the application of tebuconazole, it impoved its phenotypic appearance by reducing the infection of this disease. When tebuconazole was applied to the V2 ,it showed better result than the V1. In control there was no infection occur to the seedling as a result no lesion found. When infection occur by MoT, the number of infected plant per pot was higher in case of treatment V1P compared to V2P. As a consequences, the number of lesion was greater in V1P than V2P. After application of fungicides,both treatment V1PF and V2PF showed the lower number of infected plants and it greatly helped to lower the number of lesion per plant. Conidia formation on wheat leaves coincided with synchronous emergence of spikes under blast epidemics, and early application of foliar fungicide at this stage might reduce spike blast infection, if these conidia could act as inoculum for spike blast (Cruz *et al*. 2015). Malondialdehyde (MDA), a substance produced by membrane lipids in response to reactive oxygen species (ROS), can be used as an indicator to evaluate the degree of plasma membrane damage and oxidative stress. Due to the infection of blast disease,the content of the malondialdehyde was increased in both V1 and V2 variety that results stress condition by increasing the ROS in plants. It is one of the final products of polyunsaturated fatty acid peroxidation in the cells; for this reason, it is a widely used and reliable marker for determining the degree of injury to a stressed plant (Morales and Munné-Bosch, 2019). The more the plant is damaged, the higher its MDA content, as found in studies that focused on plant responses to abiotic and biotic stresses (Alché, 2019). That is to say, plants will generate ROS under abiotic or biotic stress conditions, thereby impairing the production of biomolecules, such as lipids, proteins, and nucleic acids, which increases the MDA content and the permeability of the plasma membrane, leading to extravasation of the content of cells. (Munnik *et al.*, 2000; Kong *et al.*, 2016). After inoculation of MoT, both V1P and V2P showed high content malondialdehyde but this rate was higher in V1P than V2P. Application of Fungicide helps to decrease the malondialdehyde content in both V1PF and V2PF treatments. Foliar pretreatment of fungicide improved the physiological process that decrease H_2O_2 and MDA content (Yuzbasioglu, 2020). Electrolyte leakage is a hallmark of stress response in intact plant cells. This phenomenon is widely used as a test for the stress-induced injury of plant tissues and ‘a measure’of plant stress tolerance (Levitt, 1972; Blum and Ebercon, 1981; Bajji *et al.,* 2002; Lee and Zhu, 2010). When a cell dies and loses the integrity of the cell membrane, electrolytes, such as K^+ ions, leak out of the cell. Thus, the amount of electrolytes leaked from a tissue is responsible for the cell death in the tissue. A simple way to quantify such electrolytes leaked from a tissue is to measure the increase in electrolytic conductivity of water that contains the tissue with dying cells. This electrolyte leakage assay has been applied to plant tissues to assess the relative quantity of cells that died in response to biotic and abiotic stresses, such as pathogen challenge, insect herbivory, wounding, UV radiation, oxidative stress, salinity, drought, cold and heat stress (Demidchik *et al.*, 2014). In present sudy, treatments V1P and V2P showed high rate of electrolyte leakage in both shoot and root due to pathogenic infection. However, application of tebuconazole not only reduced the electrolyte damage but also improved the root and shoot growth that showed in case of V1PF and V2PF treatments.In plants the production of reactive oxygen species (ROS) is a common outcome of various metabolic reactions that occur in multiple sites within a plant cell. ROS like hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet}-$), the hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$) are also produced as one of the earliest responses of plant cells to environmental stresses, and these ROS molecules can cause damage to a variety of biological processes (Halliwell, 2006; Gill and Tuteja, 2010; Das and Roychoudhury, 2014). Anjum *et al.*, 2015, found that High ROS levels can result in extensive damage to proteins, DNA, and lipids, thereby affecting normal cellular functions, which can lead to permanent metabolic dysfunction and plant death.Major sources include misfires in the electron transport chains of chloroplasts and mitochondria, the Mehler reaction such as wide variety of limited substrate oxidases, type III peroxidases, and NAD(P)H oxidases (Halliwell and Gutteridge 1999). Some of these produce H_2O_2 directly, and others only via more reactive intermediates (e.g.$^1O_2$ or $O_2\bullet-$). Broadly, these events are enhanced by stresses (Alscher *et al*. 1997; Bolwell, 1999). The oxidative burst associated with part of hypersensitive response to pathogens also cause rapid increase in the concentration of H_2O_2 (Miller *et al*., 2010). After inoculation, hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$) content increased in the plant , for that reason plants face more stress condition. Application of tebuconazole decreased the stress condition of plant to a great extent which is showed in treatments V1PF and V2PF.The enhancement of the plant antioxidative system by the fungicides significantly and considerably reduced the level of superoxide ($O_2\bullet-$) in leave (Wu and Von, *et al.* 2002). Relative water content (RWC) is probably the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. RWC is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and osmotic adjustment (Barrs and Weatherley, 1962). It indicates the actual water content to its maximum turgidity. In present study, it was observed that, plants under zero stressed condition had maintained higher RWC (V1 and V2) than severe stressed plants (V1P and V2P). After application of tebuconazole, treatments (V1PF and V2PF) showed better maintenance of higher RWC ensuring better hydration and more favourable internal water relations of tissue with a possibly higher pressure potential and showed better growth. Photosynthesis is a well-studied process in which plants convert light energy into chemical energy and transform carbon dioxide to produce organic molecules, which are then used for plant growth and development (Simkin *et al.*, 2020). Photosynthesis is strongly influenced by drought, cold, salt, heat, oxidative stress, heavy metal toxicity, and other stressors (Huang *et al.*, 2019a; Liang *et al.*, 2019; Yang *et al.*, 2020). A reduction in Chl *a*, Chl *b*, and total chlorophyll content was found under the influence of several stresses (Sidhu *et al.*, 2017; Hamani *et al.*, 2020). Similarly, environmental constraints reduce the activities of photosystems and even lead to the inhibition of photosystem I and II activities (Tan *et al.*, 2020). Stress adversely affects the chlorophyll fluorescence parameters, which indicates the reduced photosynthetic performance of plants (ALKahtani *et al.*, 2020). Plant water status and photosynthesis are governed by stomatal conductance, and it is found to be severely affected by various stressors (Faralli *et al.*, 2019). In the present study, the rate of photosynthetic pigments showed variation among various treatments.V1P and V2P faced severe stress codition due to the infection of MoT. As a result, the rate of phosynthetic pigments fall down resulting poor growth. Stress conditions destroy the chloroplast ultrastructure and lead to a decrease in chlorophyll, which results in lower photosynthetic activity (Sidhu *et al.*, 2017). The infection of blast disease, Chl *a* content decreased more in BARI Gom 26 than BARI Gom 33 compared to control plant. After application of tebuconazole, Chl *a* content was improved in both BARI Gom 26 and BARI Gom 33 compared to diseased plant. Due to the interaction with deleterious blast pathogen, plant cannot sustain its normal growth. So, the normal length of both root and shoot is decreased. In BARI Gom 26, shoot and root length decreased more than BARI Gom 33 compared to control plant. Due to the application of tebuconazole, highest shoot and root length found in BARI Gom 33 compared to blast infected plant (V2P). Similarly, in BARI Gom 26, shoot and root length also improved by the application of tebuconazole compared to blast infected plant (V1P). A considerable variation was observed in the fresh and dry weight of shoot and root of wheat seedling. Shoot fresh and dry weight in both BARI Gom 26 and BARI Gom 33 varied significantly due to the MoT infection. The highest weight found in BARI Gom 33 (V2) whereas the lowest in BARI Gom 26 (V1) variety. The result showed that tebuconazole application successfully increased the shoot and root fresh weight of both varieties. However, the rate was greater in BARI Gom 33 than in BARI Gom 26. The stress condition significantly decreased the fresh weight of root and shoot. The varietal difference for this attribute was marked under stress. In case of dry weight, effect of stress lowered the dry weight of the roots and shoot of both varieties. Application of tebuconazole successfully increased the dry weight in both varieties. So, The interactions of fungicide with stressed plants played a significant role to enhance the growth of the entire wheat plant.

CHAPTER VI

SUMMARY AND CONCLUSION

Plants are constantly suffered by a wide range of biotic and abiotic stresses. As a sessile organism, plant cannot move, thus exposed to a particular abiotic/biotic stress or a combination of both abiotic stresses/biotic stresses or abiotic and biotic stresses unitedly. Some studies revealed that pathogenic infections decreased plant tolerance to environmental stresses, while abiotic stress also made the plant susceptible to pathogenic infection. The reduction of pathogen induced oxidative damage by exogenous application of chemicals might be an effective technique. Keeping this hypothesis in focus the present studies have been conducted to explore the function of tebuconazole fungicide to improve blast diseasetolerance in wheat seedlings.

From this study, it was found that blast disease hampered the physiological and biochemical functions of wheat seedlings. The seedlings treated with MoT resulted in typical blast symptoms observed on the leaves and then most of the leaves are withering. As a result, higher number of infected plant and leaf lesion observed. The inoculation of MoT increased MDA, shoot and root electrolyte leakage and H_2O_2 and $O_2^{\bullet-}$ production, thus increased the oxidative damage. Blast induced oxidative damage inhibited the normal function of wheat seedlings by reducing leaf relative water and photosynthetic pigment contents,therefore reduced plant growth and biomass. However, this toxic effect can be alleviated by applying tebuconazole fungicide, which promotes the tolerance of blast disease. The effects of alleviation are associated with improving plant growth, biomass, relative water content and photosynthetic pigment contents. Thus, oxidative damage reduced by decreasing the production of MDA, EL and ROS production (Fig. 15).

The findings of the present study indicate the protective role of tebuconazole fungicide in regulating plant growth and improving blast tolerance in wheat plants. These findings might further assist in developing blast tolerant wheat varieties. Moreover, researchers might get information about tebuconazole fungicide as phytoprotectants for management of biotic stresses. Therefore, further investigations are needed under field conditions in different varieties of wheat to justify the tebuconazole -mediated plant tolerance mechanisms under blast disease conditions.

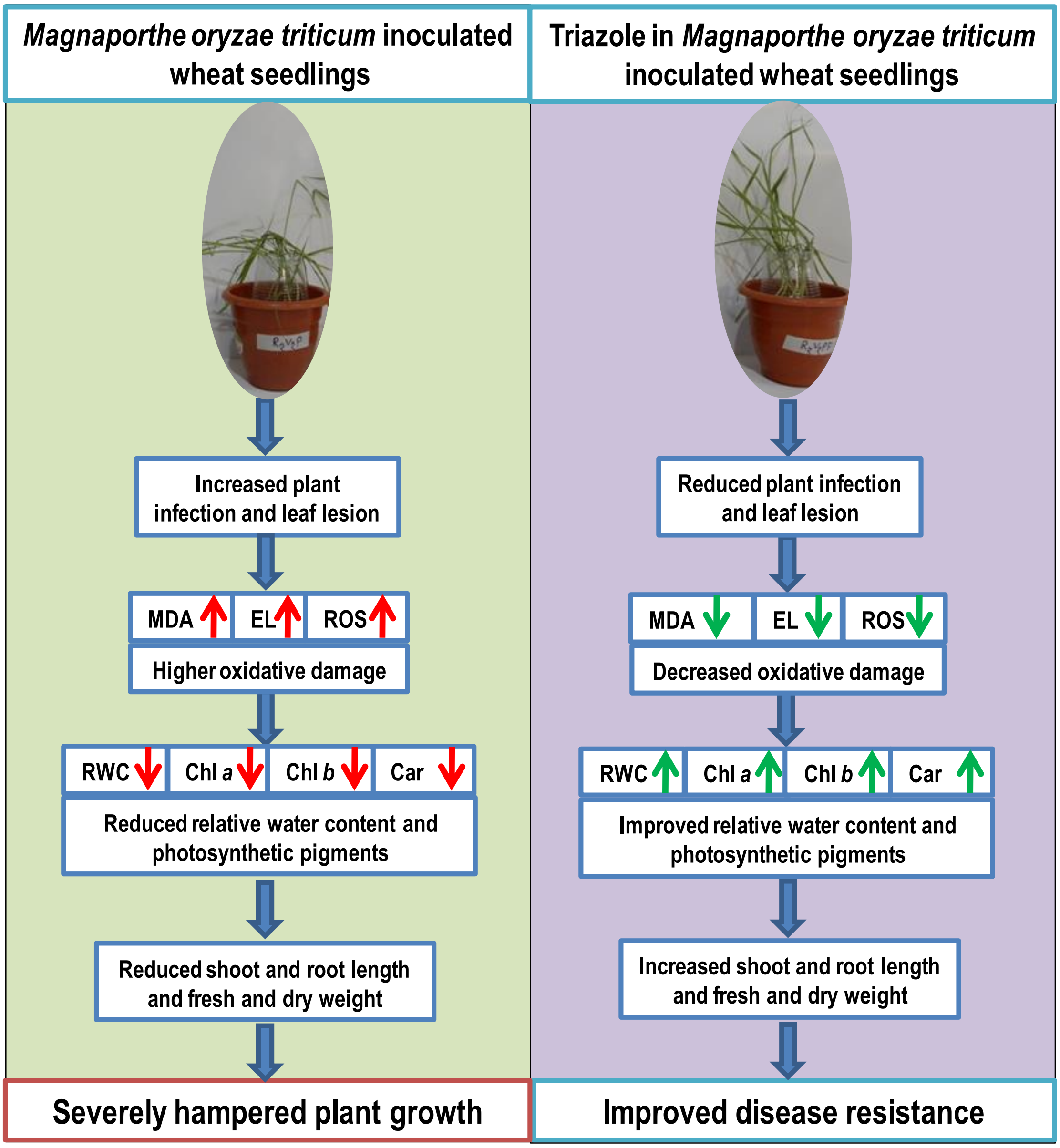

Fig. 15 Possible mechanisms of tebuconazole fungicide for improving disease resistance in wheat seedlings based on the present investigation.

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